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SOIL SCIENCE

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KEIJIRO ASO

UNIV. OF MICH.

KEIJIRO ASO

To Be Honored by Japanese Agricultural Societies

The sixtieth birthday of Keijiro Aso and his 35 years of continuous service on the faculty of the Tokyo Imperial University will be celebrated on April 7 in Tokyo at the annual meeting of the scientific agricultural societies of Japan.

Doctor Aso was born in Tokyo in 1875. He was graduated from the course in agricultural chemistry at Tokyo University in 1899 and received the degree of doctor of agriculture (Nogaku-Hakushi) upon completion of the University Hall (the post-graduate course) in 1904. In 1901, he was appointed Assistant Professor and sent by the government to study for three years the science of soils and manures in Europe and in America. He studied for one year under Professors Raman and Hiltner in München, and for two years under Professors Lemmermann, Wahnsshaffe, and Nerst in Berlin. He received instruction also from Professor Treitz in Budapest.

He attended the Second International Conference of Soil Science at Stockholm in 1910, and also served on one of the exhibition juries at the world exposition held at Turin, Italy, in 1911.

On his return to Tokyo, Doctor Aso was appointed Professor of Agricultural Chemistry and also served as an inspector of the Department of Education. Meantime he was elected President of the Chemical Society of Japan. He founded the Society of the Science of Soil and Manure of Japan and at present is president of that society as well as representative of the Japanese Section of the International Society of Soil Science.

In 1930, Professor Aso was again sent abroad by the government and attended the Second International Congress of Soil Science at Moscow and Leningrad. He was elected one of the General Committee of the International Society of Soil Science at Washington and also at Moscow. After the Congress, he visited Germany, Sweden, Denmark, France, England, and America.

Again returning to Japan, he was elected Dean of the Agricultural Faculty of Tokyo Imperial University. On his sixtieth birthday, in June, he will retire from active service at the University.

A NEW INSTRUMENT FOR SOIL SAMPLING

AASULV LÖDDESÖL

The Norwegian Peat Society, Oslo, Norway

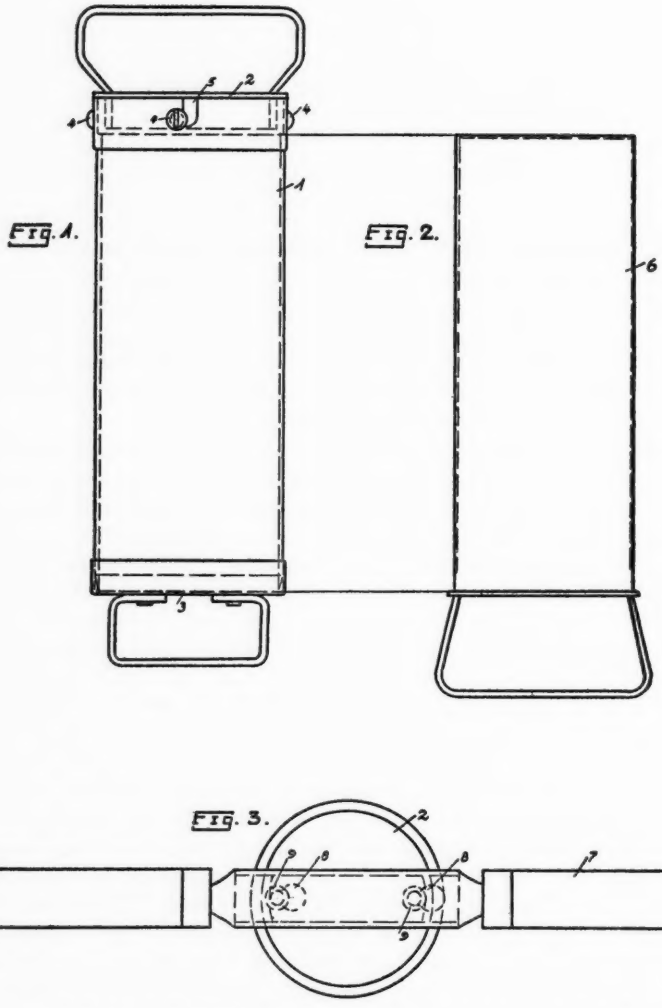
Received for publication May 2, 1934

In taking samples of soils for the determination of specific gravity and for subsequent chemical analysis, it is important that the samples should be taken vertically from the surface to a prescribed depth or from one prescribed level in the profile of the soil to another, so that the volume of the sample is exactly known. This in order to have a definite basis for an exact determination of the specific gravity and for the calculation of the content of plant nutrients per unit area of the soil, as a rule to a depth of 20 cm.

In order as far as possible to fulfill the demand for exact sampling and for a definite determination of specific gravity, the writer has constructed a special instrument for taking samples of soils predominantly organic in nature and another for mineral soils.[1]

The sampler for organic soils consists of a brass cylinder with an internal diameter of 7.98 cm., thickness of metal 2.0 mm., and height from the cutting edge to the bottom of the cylinder 20.0 cm. (fig. 1 and plate 1, no. 1). The internal volume of the cylinder is consequently exactly 1 liter, and the sample is taken to exactly the required depth, viz., 20 cm. The upper edge of the sampler has a flange 1.5 cm. in height, with an internal diameter of 8.20 cm., into which is fitted a loose lid which forms the bottom of the cylinder (figs. 1, 3 and plate 1, no. 2). The lid is provided with a fixed handle which is large enough to admit one hand. By the aid of four rivets (fig. 1 and plate 1, no. 4) which fit into four slots in the outer edge of the cylinder (fig. 1 and plate 1, no. 5), the lid is easily fastened to the cylinder. The fixed handle can be provided with a lever (fig. 3 and plate 1, no. 7) large enough for both hands if it is necessary to use more force to press the cylinder down into the soil. The loose arm consists of a U-shaped iron plate which covers the fixed handle and, in addition, is fixed by the aid of two rivets (fig. 3 and plate 1, no. 9) which fit into holes with corresponding slots in the fixed handle (fig. 3 and plate 1, no. 8). The sample cylinder is emptied by removing the lid and inserting into the cylinder a perfectly fitting, light, hollow copper cylinder (fig. 2 and plate 1, no. 6), which is provided with a bottom at one end and a handle at the other. The soil is emptied into a box, or the like, which is used for packing. In order to avoid loss of soil or moisture, if a packing-case is not at hand, a lid (fig. 1,

[1] The soil sampler can be supplied by the Central Scientific Company, Chicago, Illinois.



FIGS. 1, 2, AND 3. DIAGRAMS SHOWING THE CONSTRUCTION OF THE SOIL SAMPLER

1—Brass cylinder; *2*—lid forming bottom of cylinder; *3*—lid covering cutting edge of cylinder; *4*—rivets in cover; *5*—slots in cylinder; *6*—inner copper cylinder; *7*—lever upon which additional force can be applied to press cylinder into soil; *8*—slots in fixed handle; *9*—rivets holding loose arm to fixed handle.

no. 3) is fitted on the mouth or cutting edge of the sampler, which to a length of about 7 mm. is cone-shaped.

The sampler and its fittings are packed into a light wooden box with internal dimensions of 34 by 11 by 10 cm. and provided with a handle for easy transportation. The hollow cylinder which is used to empty the sampler is then placed inside the latter.

Solid, drawn brass tubing, a material which after 1 year's testing has proved to be very serviceable for taking samples of organic soil, is recommended for the sampler.

For hard and stony soils the sampler must be smaller and made of a harder material. The writer has therefore constructed for these soils a special sampler of rustless steel, with an internal diameter of 3.57 cm. At a depth of 20 cm., therefore, the volume of the sample is 1/5 liter. In other respects the sampler is exactly like that previously described for soils predominantly organic in nature but in emptying the cylinder, when the soil is very dry, a little T-shaped iron rod with a spade-shaped extension at the end about 5 cm. long and 1.5 cm. wide has been found practical for loosening the soil in the cylinder.

Samples are taken by making a hole in the ground with a spade and removing the vegetation so that the surface is quite level along one edge of the hole. The sample cylinder with lid adjusted and, if desired, with the lever on, is then screwed into the soil to the 20-cm. mark (which, of course, is at the same height as the bottom inside the cylinder) at a suitable distance from the edge of the hole. The spade is then inserted into the wall of the soil just beneath the cylinder, which is then taken out and emptied in the manner already described. If the soil is very loose, the spade can be inserted in the wall of the soil 20 cm. below the surface *before* the sampler is screwed down. If samples of soils are to be taken from deeper layers, suitable soil ledges or steps are made. If samples of thinner strata than 20 cm. are desired, the spade is merely placed in the wall of the soil at the desired distance below the surface or below the top of the soil ledge, and the volume of the sample can be computed from the base and the depth of insertion of the cylinder.

For packing 1.25 liter[2] tin boxes with a spring catch, or rubber bags are recommended. The latter, being quite water-proof, prevent loss of either soil or water during transportation to the laboratory. If samples of soils larger than 1 liter are needed from the same place, in the case of soils predominantly organic in nature, several l-liter samples can be taken, of course, and either larger boxes used for packing or the samples sent separately and the mixture made in the laboratory. In the case of mineral soils, five samples are taken and mixed at once. The determination of the specific gravity is then a very simple matter, for after the soil has been weighed and its water content determined we have the dry weight of the soil just as it is deposited in nature. It is obvious that this is of importance in the subsequent treatment of the analysis data obtained.

[2] One liter and a quart.

PLATE 1

THE COMPLETE SOIL SAMPLER

For explanation of numbered parts, see figures 1, 2, and 3

7
9
8
4
2
5
1
6

THE AMMONIUM CARBONATE METHOD OF DISPERSING SOILS FOR MECHANICAL ANALYSIS

AMAR NATH PURI

Irrigation Research Institute, Lahore (India)

Received for publication October 9, 1934[1]

During recent years a good deal of attention has been directed toward the methods of dispersing soils for mechanical analysis. The newer methods aim at attaining maximum dispersion so that all the compound particles are broken up into their ultimate units. Working on the original suggestion of Robinson, the International Society of Soil Science and the Agricultural Education Association (England) have adopted the use of 6 per cent hydrogen peroxide for the oxidation of organic matter, though in routine work no distinction is made between soils that require hydrogen peroxide treatment and those that do not, the hydrogen peroxide treatment being generally given to all types of soils. The soil after the hydrogen peroxide treatment is treated with dilute HCl, followed by shaking with water to which 4 cc. of *N* NaOH has been added.

In the Bureau of Chemistry and Soils method (4) the acid treatment is optional and the dispersing agent is sodium oxalate. In the other three methods; namely, the NaCl method (5), the Sudan method (2), and Troell's method (7) neither H_2O_2 nor HCl is used. In the Sudan method the dispersing agent is Na_2CO_3. This method is not suitable for pipette technique. In the NaCl method proposed by the author, the soil is treated with *N* NaCl, and after the excess has been removed with a little water, sufficient NaOH is added to make the suspension alkaline to phenolphthalein. In Troell's method the oxidizing agent is sodium hypobromite, after the removal of which the soil is treated with NaCl.

The author has shown elsewhere (5) that the most effective method of dispersing soils is to introduce Na in the exchange complex, and the NaCl method is based on that principle. It was found, however, that certain ferruginous and humus soils did not respond to this treatment, and modification of the method for those types of soils was considered necessary.

In a study on the use of ammonium carbonate as a reagent for determining exchangeable bases in soils (6) the author found that when a soil containing exchangeable Ca is treated with ammonium carbonate, the whole of the replaceable Ca is converted into $CaCO_3$, and ammonium ions take their place. This

[1] *Editor's note:* At the author's request, the order of publication of this paper and of one received May 10, 1934 has been reversed.

gave the clue to a very simple method of converting calcium soils into sodium soils. The soil is boiled with ammonium carbonate solution followed by heating with NaOH solution, when ammonia is driven off and NH_3 ions are replaced by Na in the exchange complex. The application of this technique for the mechanical analysis of soils followed as a matter of course. The use of ammonium carbonate not only proved effective for soils that responded to the NaCl treatment, but it produced maximum dispersion in ferruginous and humus soils for which the NaCl method had not proved satisfactory.

DETAILED DESCRIPTION OF THE AMMONIUM CARBONATE METHOD

From 10 to 20 gm. of soil is boiled on a rose burner with 250 cc. of *N* $(NH_4)_2CO_3$ (pure) until the volume is reduced to half. Tall beakers of 600 cc. capacity are found most satisfactory and are permanently marked at 125 and 250 cc. The beaker should be covered with a watch glass for the first 10 minutes or so when the boiling is more vigorous. If the suspension shows a tendency to froth, 10 cc. of kerosene oil or of a mixture of equal parts of kerosene oil and gasoline is added when frothing immediately subsides. When the volume of the suspension has been reduced to about one half, 4 to 8 cc. of *N* NaOH or LiOH for every 10 gm. of soil is added and the suspension brought back approximately to the original volume by the addition of hot water. The boiling is continued until the volume is again reduced to one half. The suspension needs no further treatment and is ready to be made up to the desired volume for the pipette method. The whole operation takes less than 2 hours, and the number of soils that can be treated at the same time is limited only by the number of beakers that can be heated at one time.

As regards the comparative merits of Na and Li hydroxides as dispersing agents, the author is inclined to favor the latter, which has better dispersing power than the former. Another point in favour of LiOH is that the actual weight of the chemical added to the soil is less in this case than in the case of NaOH.

For soils containing large quantities of gypsum and soluble salts the method is modified as follows:—

After the first boiling with *N* $(NH_4)_2CO_3$, about 100 cc. of 2 *N* $(NH_4)_2CO_3$ is added to the suspension and filtered through a Buchner funnel on which a filter paper is stuck with molten paraffine wax. The soil is then leached once only with 100 cc. of 0.2 *N* $(NH_4)_2CO_3$. It is then taken up with about 250 cc. of water, boiled for 10 minutes, and 4 to 8 cc. of NaOH or LiOH is added and boiling is continued till the volume is reduced to one half. The suspension is then ready for making up to the desired volume for the pipette method.

It might be mentioned that the addition of 8 cc. of *N* NaOH or LiOH is not necessary for all soils, in fact the majority of soils require only 4 to 5 cc. Approximately 1.3 cc. *N* alkali is required for every gram of clay, and 8 cc. is the upper limit required by red ferruginous, or black cotton, soils.

EXPERIMENTAL

Altogether 88 soils were examined. These comprised practically all the types found in India and were specially selected out of a larger collection so as to include the largest available number of those soils that had failed to give

TABLE 1

Clay content of ordinary agricultural soils

SOIL NO. P. C.	LOCALITY	pH VALUE	CARBONATES	CLAY	
				NaCl method	$(NH_4)_2CO_3$ method
			per cent	*per cent*	*per cent*
1	Pusa (Bihar)	8.45	37.05	11.3	13.7
4	Ranala (Punjab)	8.55	0.98	15.2	18.1
5	Ranala (Punjab)	8.77	1.64	12.3	14.8
8	Coimbator I (Madras)	8.41	0.65	25.2	25.7
10	Coimbator II (Madras)	8.71	1.08	35.6	37.0
11	Coimbator III (Madras)	8.77	2.48	32.8	31.0
17	Kalyanpur (U.P.)	8.20	0.78	11.9	17.5
19	Mandalay (Burma)	8.40	1.54	42.4	44.2
21	Kaing (Burma)	8.25	3.31	13.5	16.4
23	Myetha (Burma)	7.41	0.28	11.3	11.8
24	Meerut (U.P.)	8.59	2.02	8.0	11.3
26	Hebbel (Mysore)	8.11	0.14	22.6	20.5
28	Nagpur (C.P.)	8.38	1.37	44.6	44.6
31	S. Travancore	8.01	3.12	22.8	28.1
34	Gurdaspur (Punjab)	7.21	0.12	11.3	15.5
35	Gurgaon (Punjab)	7.98	0.12	18.3	18.3
36	Hansi (Punjab)	8.45	0.52	11.7	14.4
43	Churland (Bihar)	8.41	5.87	19.7	24.7
44	High Land (Bihar)	8.54	7.46	8.4	13.2
48	Montgomery I (Punjab)	8.55	6.24	19.8	24.8
50	Tarnab I (N.W.F.P.)	8.54	16.95	17.7	19.6
51	Tarnab II (N.W.F.P.)	8.68	17.00	12.9	13.9
52	Sabour (Bihar)	8.02	0.18	11.3	10.0
54	Montgomery II (Punj.)	8.71	3.16	3.2	6.2
55	Sakrand I (Sindh)	8.36	11.32	11.0	14.9
56	Sakrand II (Sindh)	8.53	11.20	13.1	17.5
59	Raghopur (U.P.)	8.83	0.50	10.9	12.2

maximum dispersion with the author's NaCl method. For convenience of reference the soils were grouped as follows:

1. Ordinary agricultural soils.
2. Black cotton soils.
3. Laterite soils.
4. Humus soils.
5. Alkali soils.
6. Soils containing soluble salts and gypsum.
7. International soils.

1. *Ordinary agricultural soils.*—This group included all soils which had no striking feature to impart them distinct individuality, but were picked up at random to represent an average type of the usual agricultural soils. The

TABLE 2

Clay content of black cotton soils

SOIL NO. P. C.	LOCALITY	pH VALUE (H)	CARBONATE	CLAY	
				NaCl method	$(NH_4)_2CO_3$ method
			per cent	*per cent*	*per cent*
2	Akola (Bombay)	8.21	5.22	59.3	62.8
3	Dharwar (Bombay)	7.64	0.15	62.2	63.8
13	Nagpur (C.P.)	8.53	2.06	58.9	60.4
27	Babbur (Mysore)	9.03	5.08	53.2	59.1
29	Jabbalpur (C.P.)	8.05	0.32	63.0	57.4
30	Hoshangabad (C.P.)	8.45	3.30	54.1	55.1
38	Broach (Bombay)	8.29	0.22	52.9	51.9
41	Poona (Bombay)	8.74	7.73	53.4	61.6
42	Khandesh (Bombay)	9.00	4.81	53.4	58.9
46	Baroda (Bombay)	7.63	0.36	56.4	59.0

TABLE 3

Clay content of lateritic and ferruginous soils

SOIL NO. P. C.	LOCALITY	pH VALUE (H)	CLAY	
			NaCl method	$(NH_4)_2CO_3$ method
			per cent	*per cent*
6	Dacca (Bengal)	5.29	28.4	31.6
9	Malabar (Madras)	5.76	21.6	22.8
12	Tocklai (Assam) Composit.	5.83	3.8	14.8
14	Estate Soil (Mad.)	5.37	11.1	31.5
15	Shillong (Assam)	7.71	19.4	27.6
18	Mandalay (Burma)	5.79	19.9	25.2
20	Lower Burma	5.64	6.5	15.1
22	Pyinana Soil (Burma)	6.85	15.2	17.8
25	Bhur Soil (U.P.)	7.40	4.0	5.8
32	Thuravoor (Tranvanc.)	5.17	62.9	72.5
37	Rangpur (Bengal)	7.65	13.1	17.0
40	Umareth (Bombay)	7.45	10.7	14.0
45	Bihar	6.33	27.3	32.6
49	Madhupur (Bihar)	5.72	5.9	15.3

results given in table 1 show that although NaCl method is quite satisfactory for these soils, the ammonium carbonate method gives slightly better dispersion.

2. *Black cotton soils.*—The characteristic features of these soils are their dark color, high clay content, and high base exchange capacity. They respond

to the NaCl as well as the $(NH_4)_2CO_3$ method. This will be seen from table 2, which records the clay content for these soils by the two methods along with other relevant data.

3. *Laterite soils.*—This group included all the base unsaturated red and yellow soils. The NaCl method failed to give maximum dispersion with some

TABLE 4

Clay content of humus soils by the international and ammonium carbonate methods

SOIL NO.	pH VALUE	LOSS ON IGNITION	CLAY	
			International (B)	Ammonium carbonate
		per cent	*per cent*	*per cent*
265	6.49	11.1	13.7	15.9
266	6.36	9.77	13.8	15.5
267	6.10	3.19	9.7	12.5
346	5.95	5.66	11.5	10.6
350	5.90	6.07	12.1	21.3
351	6.08	7.5	17.4	18.1
352	6.31	4.06	18.0	20.8
395	5.58	6.87	29.4	28.8
190	5.07	8.25	14.0	13.5
S.30	5.40	26.9	41.9	49.3
S.34	5.10	16.5	43.8	43.6
S.58	6.00	14.8	43.6	39.6
S.64	5.70	10.8	19.5	22.6

TABLE 5

Clay content of alkali soils

SOIL NO. P. C.	LOCALITY	pH VALUE	CARBONATE	CLAY	
				NaCl method	$(NH_4)_2CO_3$ method
			per cent	*per cent*	*per cent*
7	Punjab	9.58	5.62	21.8	26.4
39	Bombay	9.11	1.46	8.9	15.3
47	Punjab	9.98	6.60	17.1	22.4
53	Punjab	9.87	6.96	17.6	17.9
57	U.P.	10.40	0.80	4.4	7.2
58	U.P.	10.36	3.45	13.4	10.0
60	U.P.	10.19	3.45	7.4	8.7

of them. The results given in table 3 show the superiority of the $(NH_4)_2CO_3$ method over the NaCl method.

4. *Humus soils.*—This group included all those soils with which the NaCl method had failed. The values in this case are compared with the International B method which is known to give maximum dispersion with such soils. The results are recorded in table 4 and show that the ammonium

carbonate method gives as good a dispersion as the International method except in one case where it is distinctly better. The last four soils in this group were kindly supplied by Dr. Sen of Dacca and were classed as lateritic. The revelant data about these soils has been taken from the paper by Chakraborty and Sen (1). The clay content of the last four soils is on the oven-dry basis; and in the case of the remaining soils the percentage of the ignited clay fraction is given.

TABLE 6

Clay content of soils containing soluble salts and gypsum

SOIL NO.	SOLUBLE SALTS	CLAY	
		NaCl method	$(NH_4)_2CO_3$ method
	per cent	*per cent*	*per cent*
H.1	15.85	3.3	11.5
H.2	35.37	2.5	7.9
H.3	15.97	16.7	20.9
H.4	24.75	22.0	21.8
H.5	15.48	20.0	21.2
H.6	7.87	10.0	14.7
H.7	14.88	11.8	14.5
H.8	10.05	11.5	15.1
H.9	2.10	20.8	25.9
H.10	48.51	12.9	15.0
H.11	18.51	10.5	32.3
H.12	16.18	16.2	18.3

TABLE 7

Clay content of international soils

SOIL	CLAY	
	NaCl method	$(NH_4)_2CO_3$ method
	per cent	*per cent*
Rendzina soil Ceji	26.8	33.9
Podsol soil Zdâr	22.0	29.8
Soil sample from Zagreb	28.0	31.3
Budapest soil	32.5	33.8
Badob soil	66.5	64.0

5. *Alkali soils.*—The characteristic features of these soils are their high pH values and the presence of exchangeable Na. Some of them contain large amounts of free Na_2CO_3 (soils 57, 58, and 60 in table 5). Free Na_2CO_3 is leached out in the NaCl method but appears as clay in the $(NH_4)_2CO_3$ method. When the percentage of soluble salts in these soils is high the modified technique is recommended. The results given in table 5 show that the $(NH_4)_2CO_3$ method is as satisfactory for these soils as is the NaCl method. As a rule

alkali soils, unless they contain gypsum, do not present any difficulty in dispersion, and even simple shaking with water is sufficient to bring about maximum dispersion for most of them.

6. *Soils containing soluble salts and gypsum.*—It is obvious that soluble salts and gypsum would cause flocculation of the soil unless they were leached out. Besides, in the pipette method the soluble salts would appear as clay, which is not desirable. Because of its limited solubility, gypsum is leached extremely slowly. In the modified technique of the ammonium carbonate method, all the gypsum is converted into calcium carbonate and ammonium sulfate. The latter can be easily leached with a single washing. The results of a comparison between the NaCl and $(NH_4)_2CO_3$ method are given in table 6.

7. *International soils.*—These soils were received by the author some time ago, in connection with the co-operative work on the methods of dispersing soils for mechanical analysis. Some of these soils will be familiar to other workers. A comparison of the clay percentage obtained by the $(NH_4)_2CO_3$ and the NaCl methods (table 7) shows the superiority of former over the latter. A reference to the results of the co-operative work given in the *Proceedings of the International Congress of Soil Science* (3) also shows that the ammonium carbonate method gives the maximum clay obtainable by any method.

CONCLUSION

In view of the importance attached to the methods of dispersing soils for mechanical analysis by the International Society of Soil Science, it is necessary that the ammonium carbonate method be given an exhaustive trial. It can not be called too drastic because boiling is already a standard procedure in the International method. The author has attempted to include all types of soils available, but the possibility of others presenting peculiar difficulties is not remote. The ideal method that would succeed with all types of soils (and of soil scientists) may still await discovery, but the present method seems to be a step in the right direction.

SUMMARY

The ammonium carbonate method of dispersing soils for mechanical analysis has been described. It consists in boiling the soil with *N* ammonium carbonate solution and continuing the boiling after the addition of some NaOH or LiOH. The method has been shown to give maximum dispersion with all types of soils including laterite and humus.

REFERENCES

(1) CHAKRABORTY, J. N., AND SEN, A. 1932 Mechanical analysis of lateritic soils. *Indian Jour. Agr. Sci.* 2: 516.

(2) JOSEPH, A. F., AND MARTIN, F. J. 1921 The determination of clay in heavy soils. *Jour. Agr. Sci.* 11: 293.

(3) NOVAK, V. 1932 Preparation of soil samples for mechanical soil analysis. *Proc. Second Internatl. Cong. Soil Sci. Comn.* 1: 14.

(4) OLMSTEAD, L. B., ALEXANDER, L. T., AND MIDDLETON, H. E. 1930 A pipette method of mechanical analysis of soils based on improved dispersion procedure. U. S. Dept. Agr. Tech. Bul. 170.

(5) PURI, A. N. 1929 A new method of dispersing soils for mechanical analysis. *Mem. Dept. Agr. India, Chem. Ser.* 10: 209.

(6) PURI, A. N. A new method of estimating exchangeable bases in soils. To appear in *Soil Sci.*

(7) TROELL, E. 1931 The use of sodium hypobromite for the oxidation of organic matter in the mechanical analysis of soils. *Jour. Agr. Sci.* 21: 476.

A SIMPLER METHOD OF EXPRESSING THE MECHANICAL ANALYSIS OF MANY COMMON SOILS*

ROBERT L. JAMES[1]

Canterbury Agricultural College and Canterbury College School of Engineering, New Zealand

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For many years a mechanical analysis (or statement of the percentage proportions of sand, coarse silt, fine silt, coarse clay, fine clay, etc.) has been made of nearly every soil studied for agricultural purposes. The analysis is usually made by shaking up a soil with water and allowing the soil particles to settle. The sand grains, which are larger in diameter than 1/20 mm., fall in the standard cylinder in 40 seconds. At the end of an hour the silt (or particles between 1/200 mm. and 1/20 mm.) have fallen to join the sands on the bottom of the cylinder. Two hours after the cylinder has been set down, the coarse clay has fallen, and so on.

Some analysts divide the soil particles into four size groups; others, into seven. Where seven groups are used the operator waits till a given time and then withdraws a certain volume of the muddy liquid, evaporates and weighs this, and calculates the weight of soil in suspension in the whole column at that particular time. This is performed seven times and constitutes a very tedious and slow process. In 1926, Bouyoucos designed a soil hydrometer which was graduated by comparing it with 30 soils analyzed by the ordinary method, so providing a simple and accurate method, which is gaining in favor. For seven groups the hydrometer is placed in the liquid, read, and removed at six particular times.

In the course of a research on "The Identification of Soils in Civil Engineering" it became necessary to analyze mechanically a number of soils with as much accuracy as possible. The soils came from Otago, Canterbury, and Auckland Provinces and were of widely different origin. On its receipt at the laboratory each soil was air dried, passed through a $\frac{1}{8}$-inch sieve, well mixed, and then stored in an air-tight cannister. The soils referred to in this paper were those which passed through a $\frac{1}{8}$-inch sieve without any appreciable amount being retained. From four to eight samples of each soil were analyzed by the hydrometer method, allowance being made for moisture content and for temperature. Just before analysis, dispersion was effected by the standard elec-

* Pipe clays and pure river-silts do not admit of this treatment.

[1] The writer wishes to express his thanks to Professor J. E. L. Cull, professor of civil engineering at Canterbury College, Christchurch, for his interest and also for the loan of laboratory space and certain equipment.

tric stirrer method. The method of dispersion was checked many times by shaking samples end over end for 48 hours on a wheel.

When all the points for each soil were plotted on a graph of *percentage of soil in suspension* against *time of hydrometer reading*, a smooth curve was obtained.

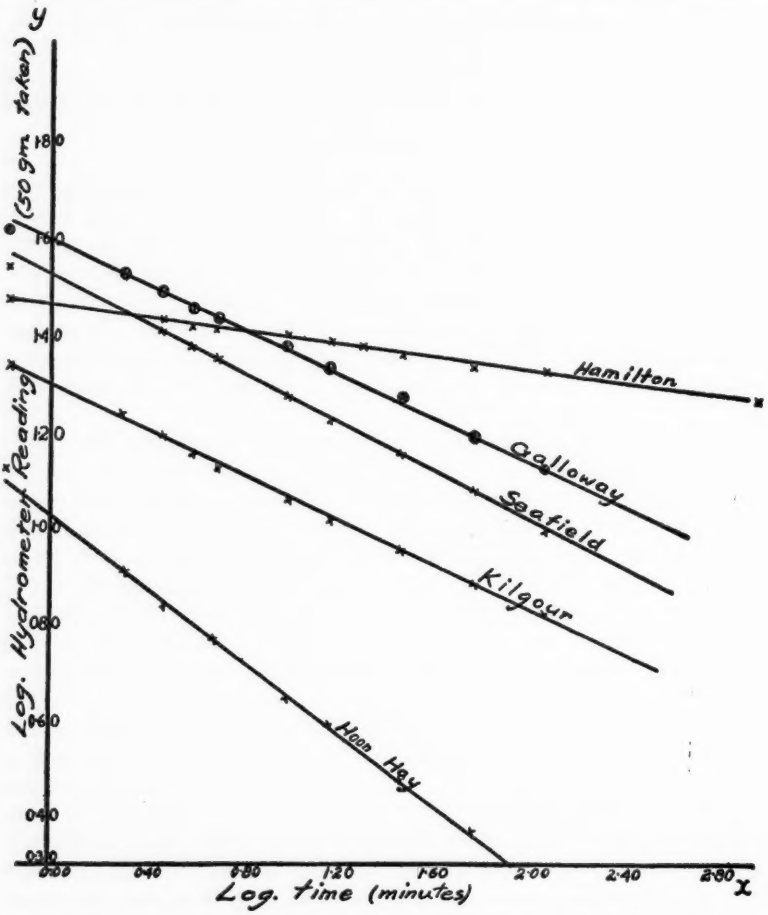

FIG. 1. SOME TYPICAL LINES FOR SOILS WHEN LOGARITHM OF TIME OF HYDROMETER READING IS PLOTTED AGAINST LOGARITHM OF HYDROMETER READING

There was a different curve for each soil, and in every case the curve was very smooth. It was then found that the smoothness of soil curves had been remarked on by G. W. Robinson (2). This encouraged the writer to plot the *logarithm of the percentage in suspension* against the *logarithm of the time of hy-*

drometer reading. In 13 cases out of 14 the result was a straight line. The exception was Glentunnel fire clay, a very hard fine-grained clay which might be counted perhaps a rock rather than a soil.

Readings taken at 40 seconds did not always conform well to the line. When the cylinder is first set down a swirling motion occurs, froth obscures the graduations, and settling occurs so rapidly that an error of a few seconds may cause a large error in the readings.

There was a different straight line for each soil, soils of similar texture having their lines close together. In geometry it is shown that such a straight line is completely described with respect to two axes of reference (lines at right angles such as those along which *log percentage in suspension* and *log time of reading* were measured), if we measure the slope of the line and measure where it cuts one of the axes. In other words, the whole result of the mechanical analysis can be expressed by two numbers for the particular soil. Typical lines are shown in figure 1.

TABLE 1

The means of eight hydrometer analyses of Hamilton soil (corrected for moisture and temperature)

TIME (T) OF READING HYDROMETER	CORRECTED HYDROMETER READING H (50 GM. TAKEN)	LOG T	LOG H
40 seconds	29.5	$\bar{1}$.824	1.470
2 minutes	28.0	.301	1.447
3 minutes	26.9	.477	1.430
4 minutes	26.1	.602	1.417
5 minutes	26.0	.699	1.415
10 minutes	25.1	1.000	1.400
15 minutes	24.3	1.176	1.386
30 minutes	22.9	1.477	1.360
1 hour	21.6	1.778	1.335
2 hours	21.3	2.079	1.328
16 hours	18.3	2.982	1.263

Example

Column 3 (table 1) is plotted against column 4 and a fair line through the points is found to be a straight line cutting the y axis (along which log hydrometer reading is measured) at the value 1.460. In geometry this value would be termed the "intercept." From antilogarithm tables 1.460 is found to be the logarithm of 28.84 (which would be the hydrometer reading at 1 minute). Since in this work 50 gm. of soil was taken, $28.84 \times 2 = 57.68$ is the percentage of material in suspension at 1 minute.

Next the slope or "gradient" of the line must be found. From the graph it will be seen that the line drops from the value 1.460 to the value 1.323 while log time changes in value from 0.000 to 2.079.

$$\therefore \text{Gradient} = \frac{1.460 - 1.323}{0.000 - 2.079} = \frac{0.137}{-2.079} = -0.066$$

That is, the numbers 57.68 and 0.066 express the mechanical analysis of Hamilton soil. The writer proposes that these two quantities be named "fine material" and "settling rate" respectively. From these two numbers the straight line can be reproduced at any time, and the amount of fine silt, coarse clay, etc. can be read off.

Theory

Co-ordinate geometry shows that the equation of a straight line, plotted by x and y co-ordinates, is $y = mx + c$ where m is a constant called the "gradient" (or tangent of the angle made with the x axis) and c is a constant called the "intercept" (or value of y when $x = 0$)

In this work $\quad \text{Log } H = m\,(\text{Log. } T) + c$

e.g. Hamilton $\quad \text{Log } H = -0.066 \text{ Log } T + 1.460$

i.e. $\quad \text{Log } H = \text{Log } (T^{-0.066}) + \text{Log } 28.84$

$$= \text{Log } (28.84\ T^{-0.066})$$

$$= \text{Log } \frac{28.84}{T^{0.066}}$$

or $\quad H = \dfrac{28.84}{T^{0.066}}$

Whence P (percentage in suspension) $= \dfrac{57.68}{T^{0.066}}$ is the equation of the settling curve for Hamilton.

TABLE 2

Complete mechanical analysis of certain soils

SOIL	FINE MATERIAL	SETTLING RATE
Hamilton	57.68	0.066
Galloway	78.72	0.234
Seafield	67.0	0.264
Horarata	63.9	0.144
Hoon Hay	21.2	0.392

An analysis expressed in terms of "fine material" and "settling rate" is readily convertible into the old nomenclature and vice versa.

The complete mechanical analysis of certain soils may be stated as in table 2.

The writer plotted the results given by Bouyoucos (1) and found that 13 out of the 15 Michigan soils gave lines which were straight within the 2 to 3 per cent which could be expected as experimental error.

The results of 14 samples of the Swan Hill Irrigation District, Victoria (3) were obtained for fine silt, coarse silt, and clay and were plotted logarithmically as before. The mid point (fine silt) deviated from the straight line joining the other two points by an amount within the limit of experimental error in each case. This was all the more remarkable since the official method used requires readings to be taken at a different depth at each pipetting. This, combined with the greater use of sieving, might be expected to mask any regular gradation in a soil. However, the official method is used very extensively.

The general result may be summarized as follows: Nearly every soil (material passing $\frac{1}{8}$-inch sieve) exhibits a regular grading in the size of its particles from coarse to fine. Further, this grading, in most cases, is such as to permit the mechanical analysis of each soil to be represented logarithmically by a straight line to an accuracy within the limits of experiment and well within the limits demanded by the agriculturist and engineer.

Application

It is easier to classify soils according to two properties than according to seven and it is easier to take two or three readings than seven. Although readings of the hydrometer taken at 5 minutes, 15 minutes, and 30 minutes would give the straight line and the complete mechanical analysis in a quarter of the usual time it is obviously unwise to shorten the analysis until a great deal of confirmatory work has been done. Exceptions are bound to occur (perhaps with pure clays or pure silts), and these may not always be easily recognizable. Again a soil could be made up to show an irregular settling curve. However, if the mechanical composition of only a quarter of the world soils (this paper records 26 out of 29 examined) can be expressed completely by the quantities "fine materials" and "settling rate" so that they are more capable of practical interpretation in their relation to tilth, water-holding capacity, suitability for drainage and irrigation, strength in foundations, porosity in dams, etc., the result is a very tangible one.

One example will be sufficient to make this clear.

The values { sand, fine silt, coarse silt, fine clay, coarse clay, organic matter, grain shape } all appear to have an effect upon foundation strength

Although each value, such as sand, grain shape, etc., can be measured, it is impossible to combine all these to predict a foundation strength. If the factors are reduced to

{ fine materials, settling rate, organic matter, shape of grains } and foundation strength,

a working rule becomes more possible. The same simplification applies to many other soil effects.

REFERENCES

(1) Bouyoucos, G. J. 1927 The hydrometer as a new method for the mechanical analysis of soils. *Soil Sci.* 23: 343–353.

(2) Robinson, G. W. 1924 The form of mechanical composition curves of soils, clays, and other granular substances. *Jour. Agr. Sci.*, 14: 626–633.

(3) Taylor, J. K., and Penman, F. 1930 A soil survey of the Woorinen settlement, Swan Hill Irrigation District, Victoria. Aust. Council Sci. and Indus. Res. Bul. 45.

A STUDY OF PHOSPHORUS PENETRATION AND AVAILABILITY IN SOILS[1]

LINDSEY A. BROWN[2]

Pennsylvania State College

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Investigation by soil scientists during the past two decades has shown that phosphorus fertilizers in general become largely unavailable to plants at the place where they come in contact with the soil. A few studies have shown that phosphorus carriers applied on permanent pasture sod penetrate only about one inch or less during the first year, and very little penetrates into the third inch even after 5 years.

The comparative effect of various fertilizer treatments on phosphorus penetration in pastures when the fertilizers are applied regularly over a period of more than a few years has not been studied. The purpose of the present study is to show the penetration of phosphorus under the aforementioned conditions and the relative penetration of rock phosphate and superphosphate under various conditions in the laboratory.

The literature dealing with phosphorus fixation dates back to 1850, when Way (19) demonstrated by simple percolation experiments that phosphate of soda dissolved in water and calcium phosphate dissolved in dilute sulfuric acid lost all of their detectable phosphates when passed through a soil. In more recent years the work of Crawley (4), Schreiner and Failyer (13), Fraps (5), Fudge (6), and Gaardner (7) show, not only that phosphorus is fixed by soils almost at the point at which it comes in contact with the soil particles, but also that clay soils fix phosphorus more rapidly than do sandy soils. Iron, aluminum, and calcium are the main elements responsible for phosphorus fixation, and the effect of nitrogen fertilizers on phosphorus fixation is due to change in soil reaction or introduced cations.

The comparison of rock phosphate and superphosphate as sources of phosphorus for plants has resulted in some conclusions which do not agree. Mooers (9), Roberts (12), and Conner (3) find that rock phosphate produces as good results as superphosphate and often better results under certain conditions.

[1] Contribution from the agronomy department, Pennsylvania State College, State College, Penna. Abstract of a thesis submitted to the Graduate School of Pennsylvania State College in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Formerly, graduate assistant in soil technology, Pennsylvania State College. Now pedologist in Conservation and Survey College at Nebraska University. The author wishes to express his appreciation to Dr. A. L. Patrick, under whose kind direction this work was conducted, and to other members of the department for their helpful suggestions and criticisms.

Bartholomew (1) and Thorne (15) have shown that superphosphate is a more efficient carrier of phosphorus than is rock phosphate.

By working superphosphate into knife grooves in old established bluegrass sod, Midgley (8) increased the yield of the grass 71.5 per cent over surface applications of the same amount of phosphorus.

The study by Brown and Munsell (2) shows that 20 months after the surface application of phosphatic fertilizers on pastures neither rock phosphate nor superphosphate treatment shows marked increases in soluble phosphorus below the 1-inch layer.

PLAN OF EXPERIMENTS

In the present study the main object was to get further information on the factors concerned in the penetration and availability of phosphorus in permanent pasture soils. The permanent pasture plots at the Snowshoe and Bradford County experiment fields offered excellent opportunity for this phase of the study. The available phosphorus and the pH of several depths in these plots were used as an indication of the most profitable additional study.

The additional study was divided into two parts; namely, first, an investigation of the factors concerned in the relative penetration of rock phosphate and superphosphate under carefully controlled laboratory conditions; the second, the determination of the effect of field applied phosphorus fertilizers on the phosphorus content of soils and plants.

METHODS OF ANALYSIS

Available phosphorus was determined on the soil samples by the method recommended by Truog (17). Throughout this discussion "available phosphorus" refers to the amount determined by this method.

The pH of the soils was determined electrometrically with the quinhydrone electrode.

A slight modification of the colorimetric method suggested by Pfeilsticker (11) was used to determine total phosphoric acid of the plants.

EXPERIMENTAL

Phosphorus penetration in field plots

Previous phosphorus penetration studies (14, 18, and 8) indicated that most of the available phosphorus applied on permanent pastures remained very near the surface. For this reason the upper 5 inches were sampled by one-half inch layers, the next 3 inches by inch layers, and the 8- to 12-inch depth was taken in two 2-inch layers. The samples were taken in November, 1932, from Snowshoe field B and Bradford County field B plots.

Soil samples from the plots were transferred to the laboratory, air dried, sieved through a 20-mesh sieve, and stored for 1 year before any analyses were made.

Table 1 shows the yields, fertilizer treatments, and amounts of available phosphorus of the Snowshoe plots. Fertilizer treatments date back 17 years prior to sampling.

Table 1 shows that most of the available phosphorus in plot 2, a permanent pasture plot which received no phosphatic fertilizer, is concentrated in the upper one-half inch of the soil.

TABLE 1

Yields, fertilizer treatments, and available phosphorus of Snowshoe field B plots

	PLOT 2	PLOT 3	PLOT 4	PLOT 5	PLOT 6	PLOT 7	PLOT 8	PLOT 9
Treatment*	Ca	Ca, P	Ca, R	Ca, P, K	None	Ca, N, P, K	Ca, M, P	Ca, M, R
Yield (lbs. per acre):†								
Weeds	596	54	101	135	443	338	115	140
Sweet clover	66	426	794	634	....	368	515	1,236
Pasture grasses	96	2,071	1,862	2,521	....	3,530	2,488	2,127
Depth, inches	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*
0–½	12	30	132	26	8	12	13	96
½–1	5	24	142	17	5	13	12	99
1–1½	5	18	134	14	3	10	9	95
1½–2	5	12	136	11	<2	9	8	84
2–2½	4	7	126	8	<2	11	7	93
2½–3	5	6	124	8	<2	10	6	81
3–3½	5	4	87	6	<2	4	3	69
3½–4	4	3	72	4	<2	3	2	59
4–4½	4	3	79	4	<2	4	<2	53
4½–5	3	4	56	5	<2	4	3	41
5–6	2	5	40	5	<2	4	3	24
6–7	2	4	39	4	<2	2	2	13
7–8	2	3	10	4	<2	2	2	4
8–10	2	3	4	4	<2	3	3	5
10–12	..	2	4	4	<2	<2	<2	5

* Ca—lime to equal Vietch lime requirement at start of experiment.
P—65 pounds P_2O_5 as superphosphate every second year.
R—260 pounds P_2O_5 as rock phosphate every second year.
K—100 pounds muriate of potash every second year.
N—300 pounds nitrate of soda every second year.
M—4 tons manure every second year.
† Average annual yield (air dry basis) according to White and Holben (20).

Plot 3 shows that very little of the superphosphate remains available and none of it penetrates deeper than 3 inches. It is impossible to say how much, if any, of the phosphorus penetrated, since the first application was harrowed in. Under these circumstances it is probable that phosphorus would be worked into the first 3 inches mechanically.

Comparison of plot 3 and 4 shows the remarkably larger amounts of available phosphorus on the rock phosphate treated plot when compared to the superphosphate treated plot. Likewise available phosphorus has penetrated to a depth of more than 7 inches where applied as rock phosphate.

TABLE 2

pH of soil samples from Snowshoe field B plots

DEPTH	PLOT 2	PLOT 3	PLOT 4	PLOT 5	PLOT 6	PLOT 7	PLOT 8	PLOT 9
inches								
0–½	5.89	5.55	5.97	5.36	4.75	5.68	5.86	5.89
½–1	6.16	5.66	6.14	5.43	4.56	5.66	5.78	6.02
1–1½	5.87	5.88	6.39	5.45	4.58	5.63	5.79	6.11
1½–2	5.87	5.95	6.42	5.60	4.56	5.67	6.11	6.23
2–2½	6.03	5.82	6.39	5.51	4.49	5.68	6.13	6.22
2½–3	5.75	5.66	6.54	5.31	4.60	5.77	5.98	6.31
3–3½	5.67	5.49	6.46	5.21	4.70	5.76	6.06	6.09
3½–4	5.43	5.23	6.44	5.17	4.72	5.73	6.05	6.16
4–4½	5.42	4.95	6.23	5.00	4.74	5.69	5.74	6.03
4½–5	5.25	5.12	5.97	5.22	4.75	5.53	5.60	5.92
5–6	5.05	4.91	5.73	5.06	4.79	5.40	5.59	5.81
6–7	4.95	4.91	5.86	4.89	4.85	5.28	5.54	5.43
7–8	5.05	4.73	5.44	4.93	4.93	5.07	5.30	5.18
8–10	4.74	4.58	5.15	4.67	4.72	4.89	5.09	4.94
10–12	....	4.53	4.72	4.61	4.69	4.73	4.94	4.79

TABLE 3

Available phosphorus and pH of plots 1, 2, 3 and 4 of field B at the Bradford County experiment

	PLOT 1	PLOT 2	PLOT 3	PLOT 4
Treatment.....	None	Lime	Superphosphate	Lime + superphosphate
Yield (lbs. per acre):*				
Weeds.....	1,519	722	1,261	351
Sweet clover.....	23	858	.....	828
Pasture grasses.....	162	1,402	506	1,831
Depth, inches	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*
0–½	29	18	80	70
1–1½	7	6	40	44
2–2½	3	5	18	23
3–3½	3	4	7	11
4–4½	4	4	4	5
10–12	3	3	3	3
	pH	*pH*	*pH*	*pH*
0–½	5.70	6.51	5.58	5.95
1–1½	5.08	6.61	4.96	5.94
2–2½	5.01	6.40	4.92	5.81
3–3½	5.00	6.10	4.79	5.68
4–4½	5.02	6.02	4.77	5.59
10–12	5.07	5.15	4.65	4.93

* Average annual yield (air-dry basis) according to White and Holben (20).

Plots 8 and 9 produced higher yields than the corresponding plots 3 and 4, which did not receive manure, but the available phosphorus did not penetrate deeper in plots 8 and 9 than where manure was not used. Available phosphorus was somewhat lower in these plots than in plots receiving no manure.

This may be caused by the absorption of more phosphorus by the plants on plots receiving manure.

These results agree well with part of the results by Brown and Munsell (2). They found a great deal more available phosphorus in plots treated with rock phosphate than in those treated with superphosphate. However, they found no more penetration of phosphorus from rock phosphate than from superphosphate. The greater penetration of available rock phosphate shown in the present study may be caused by a longer term of fertilizer treatment than that represented in Brown and Munsell's work.

Apparently there is no significant difference in phosphorus penetration and availability between plots 5 and 7. This is contrary to the findings of Midgley (8), who reports greater phosphorus penetration where sodium nitrate is applied with superphosphate than where sodium nitrate is not applied.

Since phosphorus availability is often correlated with soil reaction, the pH of the soil samples from Snowshoe was determined. Results of these determinations are given in table 2.

Table 2 shows that the only consistent difference between plots that received limestone is the more alkaline reaction of the plots receiving their phosphorus in the form of rock phosphate.

Results of the determination of available phosphorus and of pH of the Bradford County soil samples verified the correctness of the results on the Snowshoe soil samples.

Table 3 shows the yields, fertilizer treatment, available phosphorus, and pH of plots 1, 2, 3, and 4 of the Bradford County field B experiment. The data indicate that there is no significant difference in the available phosphorus content of a superphosphated soil when limed and when not limed. Also practically all of the available phosphorus remains in the upper $3\frac{1}{2}$ inches of the soil. The consistently more acid reaction of plots receiving superphosphate over those receiving no phosphorus is the most significant fact shown by the pH values in table 3. This may be caused by the crops on phosphorus treated plots absorbing more calcium and other bases from the soil, which leaves the soil more acid.

Laboratory percolation studies

The results shown in table 1 indicate that rock phosphate solubility in soils and the crop response from it can not be correlated. That is, although rock phosphate plots show more available phosphorus than do superphosphate plots, crop response shows that superphosphate is the more efficient fertilizer. Additional information comparing rock phosphate and superphosphate therefore seemed desirable.

The laboratory percolation tests were conducted in glass percolation tubes. The bottom hole of each tube was covered with cheesecloth, and pulped filter paper in water was placed on top of the cloth to form a tight fitting filter pad (fig. 1, D). Three hundred grams of stock soil (fig. 1, C) was placed in the

tube. The upper filter pad where present (fig. 1, B) was made by pouring filter paper in water on top of the soil.

The stock soil used in all percolation tests was Morrison sand, subsoil. This soil is almost free of available phosphorus, water penetrates it rapidly, there is very little exchangeable calcium in it, and it is a residual soil formed from a high iron content, coarse-grained sandstone.

In most of the tests four times as much phosphorus was applied in the form of rock phosphate as was applied as superphosphate. These are the amounts applied on the Snowshoe field B plots and many other field tests of the two phosphorus carriers.

The fertilizer was mixed with 40 to 50 gm. of the stock soil and clean quartz sand (fig. 1, A). Distilled water for leaching was supplied through a system of syphons. The water dripped on the filter paper on top of the soil at a con-

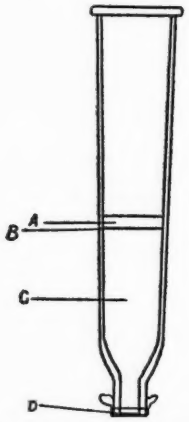

FIG. 1. PERCOLATION TUBE SETUP USED IN LABORATORY PENETRATION TESTS

trolled rate. The object of this was to make the water application as near like a continuous rain as possible. The penetration of phosphorus by this method was no doubt greater than under field conditions. However, comparative penetration between treatments was considered more like that obtained under field conditions than if the soil were flooded by additions of several inches of water at a time.

All percolation tests were run in duplicate. The averages of duplicates are reported here. The object of the first percolation test was to determine whether phosphorus penetrated the soil entirely as a solution or partially as a suspension of small particles. In this test half of the percolation tubes were fitted with filter pads between fertilizer and stock soil (fig. 1, B) and the others had no obstruction to the movement of the phosphorus carrier in suspension. Distilled water was slowly dripped on the soils, as previously described, for

about 144 hours. At the end of this time 65 inches of water had passed through the soils. The results of this first percolation test are shown in table 4.

Table 4 shows that preventing the movement of superphosphate in suspension has very little effect on the penetration of phosphorus that is available. In the rock phosphate treated soils, however, roughly three times as much available phosphorus had penetrated below the layer in which it was mixed where the filter pad was absent as where it was present. Analysis of the filter pads showed that immaterial amounts of phosphorus were absorbed by them. These data would lead us to believe that probably a large part of the penetration of rock phosphate at Snowshoe was caused by the fertilizer being carried down as particles in suspension.

TABLE 4

Available phosphorus and pH of Morrison subsoil from percolation tubes after leaching with 65 inches of distilled water

TREATMENT*	SURFACE† 50 GM.		NEXT 100 GM.		2ND 100 GM.		3RD 100 GM.		LEACHATE
	mgm. P	*pH*	*mgm. P*	*pH*	*mgm. P*	*pH*	*mgm. P*	*pH*	*mgm. P*
None	0.18	5.59	0.25	5.45	0.30	5.35	0.26	5.34	None
Superphosphate 400‡	1.57	5.76	1.42	5.67	0.75	5.61	0.16	5.40	0.008
Rock phosphate 915‡	17.03	6.35	0.56	5.99	0.23	5.19	0.23	5.22	0.070
Superphosphate 400	1.75	5.49	1.35	5.52	0.84	5.46	0.46	5.39	0.050
Rock phosphate 915	13.00	6.33	1.70	5.94	0.52	5.39	0.38	5.27	0.100
Superphosphate 400, $NaNO_3$ 320	1.65	5.80	1.76	5.58	0.80	5.49	0.48	5.31	None
Superphosphate 400, $(NH_4)_2SO_4$ 233	1.38	5.52	1.42	5.51	0.71	5.46	0.43	5.39	0.030
Rock phosphate 915, $NaNO_3$ 320	12.90	6.12	0.76	5.76	0.40	5.35	0.25	5.27	0.190
Rock phosphate 915, $(NH_4)_2SO_4$ 233	14.60	6.30	0.69	5.56	0.30	5.42	0.18	5.11	0.213

* Fertilizer used and pounds per acre.

† Fertilizer mixed with this layer prior to leaching.

‡ Filter pads placed just under the surface 50 gm.

The percolate from sodium nitrate plus superphosphate treated soil was considerably lower in phosphorus than the percolate from soil receiving superphosphate only. Sodium nitrate had little, if any, effect on the penetration of rock phosphate but allowed more to be carried into the percolate. These data do not agree closely with the findings of Midgley (8), who has shown superphosphate to be considerably more soluble when applied with sodium nitrate than when applied alone.

After the comparisons of rock phosphate and superphosphate in soils made thus far, rock phosphate was applied in sufficient quantities to furnish four times as much total phosphoric acid as the superphosphate applications. Also the laboratory percolation tests were on soils of pH about 5.4. It seemed advisable, therefore, to test the effect of some treatments which contained the same amounts of phosphorus from superphosphate and from rock phosphate. Table 5 gives the results of this percolation study.

Superphosphate, in comparison to rock phosphate, has a great deal more phosphorus soluble at a pH near neutrality than at pH 5.5, as shown in tables 4 and 5. When superphosphate was applied to equal the phosphoric acid strength of the rock phosphate application in a nearly neutral soil, about 50 per cent more phosphorus was available in the rock phosphate soil than in the superphosphate soil after leaching both with 50 inches of distilled water. However, table 5 shows that superphosphate penetrates the soil much more readily than does rock phosphate under these conditions.

The effect of soil reaction on superphosphate and rock phosphate penetration and availability in a soil was tested by bringing the Morrison sand subsoil of pH 5.03 to various pH values by the use of lime or sulfuric acid. The fertilizer was mixed with 40 gm. of the stock soil containing 20 per cent white sand. The mixture was applied on the surface of the soil in the percolation tubes. The soil was continuously leached with distilled water for 4 days,

TABLE 5

Available phosphorus and pH of limed Morrison subsoil from percolation tubes after leaching with 50 inches of distilled water

TREATMENT*	SURFACE† 45 GM.		NEXT 100 GM.		2ND 100 GM.		3RD 100 GM.		LEACHATE
	mgm. P	*pH*	*mgm. P*	*pH*	*mgm. P*	*pH*	*mgm. P*	*pH*	*mgm. P*
None	0.09	6.82	0.30	7.06	0.30	7.38	0.30	7.42	None
Superphosphate 400	0.30	6.38	1.10	6.57	1.20	7.18	1.00	7.10	0.090
Superphosphate 1,600	1.90	6.17	3.00	6.76	3.70	6.94	3.20	7.25	0.188
Rock phosphate 915	13.10	6.86	4.30	7.46	0.60	7.40	0.40	7.39	None

* Fertilizers and pounds per acre.

† All fertilizer was mixed with this layer which was 27 per cent limed Morrison subsoil and 73 per cent washed white sand.

during which 48 inches of water was passed through the soil. Data resulting from the study are shown in table 6.

Reaction near neutrality seems to favor phosphorus penetration and availability under the conditions of the study. In the very acid soil and in the soil containing free carbonates, phosphorus penetration from both superphosphate and rock phosphate was greatly depressed. Both rock phosphate and superphosphate made the greatest penetration in a soil almost saturated with calcium but with no free carbonates present.

The reason for more available phosphorus at pH 7.9 than at any other reaction may be explained by the low solubility of iron and aluminum and the little free calcium at this reaction.

Vegetative tests

All data accumulated thus far seemed to indicate that rock phosphate should supply the plant with phosphorus at least as well as does superphos-

TABLE 6

Available phosphorus and pH in limed and acid treated Morrison subsoil from percolation tubes after leaching with 49 inches of distilled water

TREATMENT*	SOIL†	SURFACE‡ 40 GM.		NEXT 100 GM.		2ND 100 GM.		3RD 100 GM.		P IN TOTAL LEACHINGS (48 IN.)
	pH	*p.p.m. P*	*pH*	*p.p.m. P*	*pH*	*p.p.m. P*	*pH*	*p.p.m. P*	*pH*	*mgm.*
None	3.77	None	7.01	0.36	5.06	0.28	4.54	0.24	4.44	None
Superphosphate 400	3.77	1.08	4.98	2.52	4.89	1.24	4.85	0.94	4.73	None
Rock phosphate 915	3.77	9.50	5.56	2.66	5.13	0.88	4.57	0.76	4.47	None
Superphosphate 400	6.37	0.76	5.40	1.46	6.22	1.62	6.37	1.36	6.45	1.320
Rock phosphate 915	6.37	12.75	7.02	4.00	7.02	0.72	6.81	0.56	6.54	0.110
Superphosphate 400	7.90	0.53	6.88	1.88	7.97	2.02	8.19	1.96	8.19	2.620
Rock phosphate 915	7.90	10.70	8.10	4.20	8.21	1.30	8.25	1.34	8.25	0.265
None	8.32	0.15	8.06	0.44	8.28	0.44	8.31	0.40	8.31	0.095
Superphosphate 400	8.32	0.64	8.17	1.90	8.35	1.40	8.35	1.00	8.35	0.762
Rock phosphate 915	8.32	1.17	8.34	0.66	8.34	0.54	8.29	0.50	8.29	0.127

* Fertilizer added and pounds per acre.

† pH of stock soil at beginning of percolation test after treatment with lime or sulfuric acid.

‡ All fertilizer was mixed with this layer.

TABLE 7

Results of analyses of soils and young plants from phosphate fertilizer test plots on which the Pennsylvania 4-year rotation is used

PLOT NUMBER	TREATMENT*	SUM OF 4 YEARS' YIELD OF CROPS†	CROP SAMPLED	P_2O_5 IN PLANTS‡	SOIL PROPERTIES	
					Available P	pH
		lbs.		*per cent*	*p.p.m.*	
1	L, P, K, superphosphate	13,809	Wheat	0.640	20	5.81
2	L, 2P, K, rock phosphate	10,476	Wheat	0.384	93	6.08
27	L, manure, + P, superphosphate	16,589	Wheat	0.941	30	6.16
28	L, manure, + 2P, rock phosphate	14,491	Wheat	0.821	69	6.23
32	L, manure, + 4P, rock phosphate	15,602	Wheat	0.878	281	6.17
1	L, P, K, superphosphate	13,809	Hay	0.183	22	5.81
2	L, 2P, K, rock phosphate	10,476	Hay	0.184	41	6.62
27	L, manure, + P, superphosphate	16,589	Hay	0.246	15	6.30
28	L, manure, + 2P, rock phosphate	14,491	Hay	0.208	45	6.05
32	L, manure, + 4P, rock phosphate	15,602	Hay	0.203	63	7.15

* L—lime to meet Vietch lime requirement.
P—48 pounds phosphoric acid per acre applied on corn and wheat.
K—50 pounds potash per acre applied on corn and wheat.
Manure—6 tons per acre.
Superphosphate—16 per cent superphosphate applied on corn and wheat.
Rock phosphate—28 per cent brown rock phosphate.

† From data by Noll, Gardner, and Irvin (10).

‡ On oven-dry basis.

phate. Since there is abundance of evidence that rock phosphate does not usually support crop growth as well as does superphosphate, further experiments were planned in an effort to determine why the solubility and plant response tests of these phosphatic fertilizers when applied to soils do not agree.

Samples of plants from certain phosphorus treated plots were obtained, dried, ground to pass a 1-mm. sieve, and analyzed for total phosphorus. Soil samples from the same plots were tested for available phosphorus and acidity. Table 7 gives the results of these analyses. These data indicate that in general there is a tendency for young plants grown on soils fertilized with superphosphate to have a higher phosphoric acid content than do plants on rock phosphate treated soil. Also hay, which is largely clover in this case, was as high in phosphoric acid where rock phosphate was used as where superphosphate was used, when manure was not applied. This might be explained by Truog's (16) theory on the ability of plants to absorb nutrients. He believes clover plants absorb large amounts of calcium, which tends to make the rock phosphate more soluble. Data presented here, however, are not held out as proof of the theory.

In all cases available phosphorus was greater in the soils receiving rock phosphate than in those receiving superphosphate. In general the rock phosphate plots were more alkaline than the superphosphate plots. This is in entire agreement with previous data.

SUMMARY

Amounts of available phosphorus at 15 depths in the upper foot of permanent pasture plots were determined by the Truog method. The data show that biennial surface applications of superphosphate penetrate not more than 2 or 3 inches and perhaps much less in 16 years. Rock phosphate applied in the same manner penetrated more than 7 inches. Eight years after the last application of rock phosphate and three years after the last superphosphate application the rock phosphate pasture contained 188 pounds per acre of available phosphorus; and the superphosphate plot, 18 pounds per acre. Rock phosphate applications contained four times as much phosphorus as did superphosphate applications.

Laboratory percolation studies show that more phosphorus is available in rock phosphate treated soils of wide range of pH and treated with various nitrogen carriers than in the same soils treated with superphosphate. Rock phosphate penetrated more rapidly than superphosphate in an acid soil, but the reverse was true in alkaline soils. Ammonium sulfate or sodium nitrate speeded the penetration of rock phosphate more than that of superphosphate, especially in an acid soil.

The field and laboratory penetration studies all show more soluble phosphorus in rock phosphate treated soils than in superphosphate treated soils. In most field tests in which a comparison has been made of superphosphate

and rock phosphate, however, superphosphate has proved the better phosphorus fertilizer.

REFERENCES

(1) BARTHOLEMEW, R. P. 1928 The unavailability of phosphorus in rock phosphate to some southern crops. *Jour. Amer. Soc. Agron.* 20: 913–920.

(2) BROWN, B. A., AND MUNSELL, R. I. 1933 Pasture investigations (second report). Penetration of surface applied lime and phosphates in the soil of permanent pastures. Storrs Agr. Exp. Sta. Bull. 186.

(3) CONNER, S. D. 1930 The availability of phosphates. *Amer. Fert.* 72 (8): 16–18.

(4) CRAWLEY, J. T. 1902 Fixation of phosphoric acid in the soil. *Journ. Amer. Chem. Soc.* 24: 1114–119.

(5) FRAPS, G. S. 1922 The fixation of phosphoric acid by the soil. Tex. Agr. Exp. Sta. Bul. 304.

(6) FUDGE, J. F. 1928 The influence of various nitrogenous fertilizers on the availability of phosphate and potassium. Ala. Agr. Exp. Sta. Bul. 227.

(7) GAARDNER, T. 1931 The binding of phosphoric acid in soil. (Trans. title abst. in *Chem. Abst.* 26: 1054.) *Kjemi Bergvesen* 11: 58.

(8) MIDGELEY, A. R. 1931 The movement and fixation of phosphates in relation to permanent pasture fertilization. *Journ. Amer. Soc. Agron.* 23: 788–798.

(9) MOOERS, C. A. 1931 Increasing the profits from phosphates for Tennessee soils. Tennessee Sta. Circ. 34.

(10) NOLL, C. F., GARDNER, F. D., AND IRVIN, C. J. 1930 A field test of different sources of phosphorus. Penna. State Col. Exp. Sta. Bul. 252.

(11) PFEILSTICKER, K. 1930 Die Anwendung der colorimetrischen Phosphorsäurebstimmung beider Keimpflanzenmethode nach Neubauer. *Ztschr. Analyt. Chem.* 82: 276–284.

(12) ROBERTS, G. 1930 Effect of associated soil treatments on the availability of applied phosphates to field crops on different soil types. *Jour. Amer. Soc. Agron.* 22: 489–497.

(13) SCHREINER, O., AND FAILYER, G. H. 1906 The absorption of phosphates and potassium by soils. U. S. Dept. Agr. Bur. Soils Bul. 32.

(14) STEPHENSON, R. E., AND CHAPMAN, H. D. 1931 Phosphate penetration in field soils. *Jour. Amer. Soc. Agron.* 23: 759–770.

(15) THORNE, C. E. 1924 Raw rock versus acid phosphate Ohio Agr. Sta. Mo. Bul. 9.

(16) TRUOG, E. 1922 The feeding power of plants. *Science* 56: 294–298.

(17) TRUOG, E. 1930 The determination of the readily available phosphorus of soils. *Jour. Amer. Soc. Agron.* 22: 874–882.

(18) WALSTER, H. L. 1926 Studies on the use of raw rock phosphate as a supplement to rotted manure on the Fargo clay. N. D. Agr. Exp. Bul. 198.

(19) WAY, J. T. 1850 On the power of soils to absorb manure. *Jour. Roy. Agr. Soc.* (Ser. I) 11: 313–379.

(20) WHITE, J. W., AND HOLBEN, F. J. 1925 Development and value of Kentucky blue grass pastures. Penna. State Agr. Exp. Sta. Bul. 195.

THE EFFECT OF NITRATE-NITROGEN ON THE CARBOHYDRATE METABOLISM OF INOCULATED SOYBEANS[1]

F. S. ORCUTT AND P. W. WILSON

University of Wisconsin

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A number of investigations indicate that closely associated with the carbohydrate-nitrogen balance in legumes is the process of nitrogen fixation; the nitrogen available to the plant is dependent, at least in part, upon the fixation process, which in turn is thought to be directly associated with available carbohydrate for protein formation and for energy requirements of the bacteria in the nodule.

It has been known for many years (3) that the production of nodules may be inhibited by the presence of various inorganic nitrogen compounds, and recently (4) a number of organic nitrogen compounds have been shown to produce the same effect. A mass of data on this detrimental effect, especially by nitrates, has been accumulated without throwing much light upon the actual mechanism of either the fixation process or its inhibition. Hopkins and Fred (4) postulate as a modification of Maze's theory, that a well-balanced carbon-nitrogen ratio is maintained in plants receiving combined nitrogen so that the concentration of carbohydrate available to the bacteria is insufficient for normal growth and energy requirements. This assumption is supported by Strowd's (9) data in which a marked reduction of sugars is accompanied by the presence of nitrate in the sap. These data, however, are very meager. The purpose of the present experiments was to make a more detailed examination of the effect of nitrates on the sugar level in the sap of inoculated soybeans.

EXPERIMENTAL

Four experiments were made in which soybean seed, of the Manchu variety, was sterilized (5) and grown in 2-gallon pots in sterile, nitrogen-free pit sand. Each pot, containing 10 plants, was inoculated with an effective strain of *Rhizobium japonicum* (in the fourth experiment the plants receiving nitrate were left uninoculated). The plants were watered with distilled water and nitrogen-free Crone's solution (1). Nitrate in five or six concentrations was added to each pot three times a week for 4 weeks, so that the nitrate concentration was held at a fairly uniform level during the first 4 weeks of growth; 0

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to 300 mgm. of combined nitrogen was added per week. The nitrate salts used in experiments 1 to 4 were respectively:—calcium, sodium, potassium, and ammonium.

The pH was determined on the plant sap by the quinhydrone electrode. Nitrate-nitrogen was determined on the fresh tissue by a modification of the phenol-disulfonic acid method (2). Reducing sugars were determined on the fresh tissue by 80 per cent alcoholic extraction. After evaporation of the alcohol to a small volume the sugars were determined by a modification of the Shaffer-Hartman micro method (8).

DATA AND DISCUSSION

The inoculated plants receiving no nitrate went through the usual nitrogen starvation period. At about the third week fixation commenced as evidenced by greener leaves and increased growth. The plants receiving 15 and 30 mgm. nitrogen per week had green leaves and were similar to the 0 series at the

TABLE 1

Effect of $Ca(NO_3)_2$ and $NaNO_3$ on pH of plant sap

ROOTS				TOPS			
NO_3-N added weekly for 4 weeks	$Ca(NO_3)_2$ 5 weeks	$Ca(NO_3)_2$ 6 weeks	$NaNO_3$ 6½ weeks	NO_3-N added weekly for 4 weeks	$Ca(NO_3)_2$ 5 weeks	$Ca(NO_3)_2$ 6 weeks	$NaNO_3$ 6½ weeks
mgm.	*pH*	*pH*	*pH*	*mgm.*	*pH*	*pH*	*pH*
0	6.00	....	5.83	0	5.77	....	5.96
35	6.03	5.97	6.42	35	5.88	5.94	5.98
70	6.04	5.98	6.31	70	5.91	5.94	6.00
140	5.97	5.94	6.42	140	5.86	5.90	6.04
280	6.30	6.08	6.47	280	5.88	6.00	6.09

4½ week harvest. The plants of the 70 mgm. series had a leaf area and plant size somewhat larger than those of the 0, 15, or 30 series. The plants receiving 150 mgm. of nitrogen similarly had large leaf areas and plant size. At an addition of 300 mgm., however, the plants began to show an excess of nitrate—occasional leaf curling.

Since the same general indications were apparent in each of the four experiments, only representative cases will be given and discussed.

pH of the plant sap. When nitrate is applied to plants a certain concentration may be reached which is definitely toxic as evidenced first by leaf curling and later by a dwarfed plant. One of the factors involved is the unused cation of the salt. In the case of Ca, K, and NH_4 salts, where the plant utilizes the cation in its metabolism, the toxic effect is somewhat counteracted. With sodium nitrate, however, an accumulation of sodium is to be expected because of the inability of the plant to utilize this ion. Such a condition raises the pH of the sap to a marked degree. This is shown in table 1, which gives

a comparison of pH values at the different levels of nitrate between plants receiving sodium nitrate and those receiving calcium nitrate. There is no particularly significant rise except in the roots of plants receiving the sodium salt; at the lowest concentration of sodium nitrate added, the pH rises 0.6 higher than in inoculated plants receiving no nitrate.

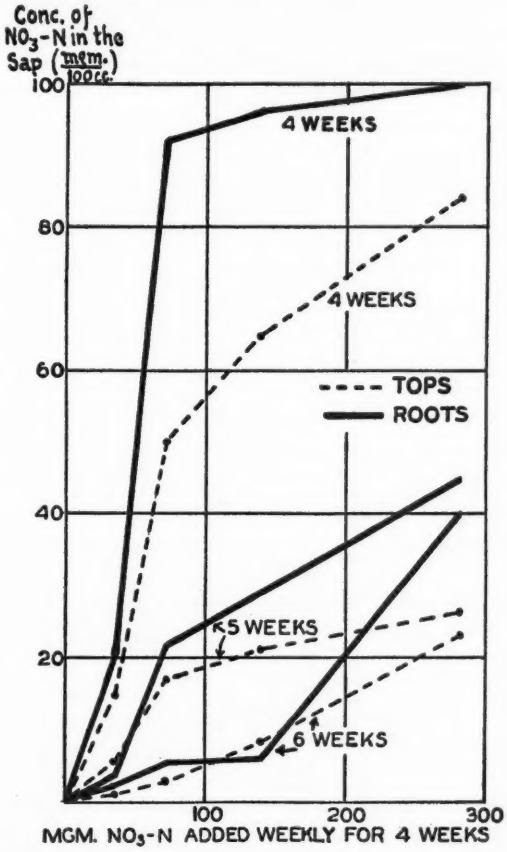

FIG. 1. COMPARISON OF NITRATE IN THE SAP TO NITRATE ADDED

Nitrate concentration in the sap. Although the concentration of nitrate in the sap is not strictly proportional to that added, as pointed out by Strowd (9), it builds up rapidly and in general approaches a direct proportion in the higher concentrations as shown in figure 1. At 4 or 5 weeks (fig. 1) the lowest concentration of nitrate added has a disporportionately low value in the sap. The nitrogen was apparently utilized more or less completely by combining with carbohydrate to form protein. In the next set of plants (at an addition

of 70 mgm. per week) the concentration of nitrate in the sap rises abruptly, apparently because of an excess over that required by the available carbohydrate. The last nitrate is added at 4 weeks, and as the stored nitrate-nitrogen is used up, all of the concentrations in the sap tend to fall to lower values at 6 weeks, and are then proportional to the nitrogen added.

Soluble sugar in the sap. The addition of nitrate considerably lowers the reducing sugar content of plant sap (fig. 2). The effect may be divided into three phases depending upon the concentration of nitrate used. These data and their interpretation are as follows:

1. A small concentration of nitrate (15 to 30 mgm.) in the substrate causes an immediate drop in the reducing sugars in the sap. This indicates that the nitrogen added in addition

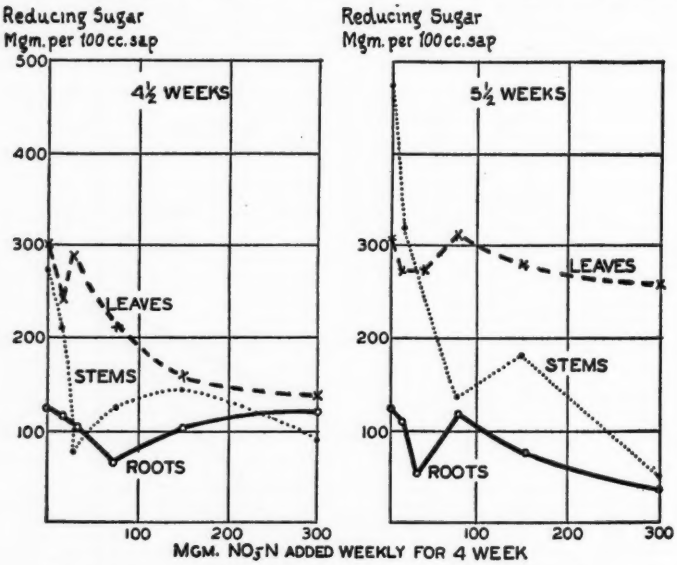

FIG. 2. EFFECT OF NITRATE ON GLUCOSE CONTENT OF PLANT SAP

to that being fixed in the nodules combines with the available sugar to form protein; the sugar level in the sap is thereby reduced and a minimum point is formed on the sugar curve.

2. As the concentration of nitrate is increased to a concentration just below that which will inhibit nodule production (70 mgm.), the size and leaf area of the plant are increased. This turns the sugar curve upward to form a maximum point. This increased photosynthetic capacity allows a partial recovery of the sugar concentration in the sap, since not enough nitrate is present for the utilization of the additional sugar formed.

3. As the nitrate concentration is still further increased, and nodule production is inhibited, the reducing sugar concentration goes down to low values at the highest concentration of nitrate tolerated by the plant. This further lowering is probably due to the increased consumption of glucose in protein formation.

This same general phenomenon, as plotted in figure 2, was also observed for sucrose. The sucrose content of the sap was approximately 50 per cent of

the soluble sugars; the sucrose graphs for the first four weeks were very similar to those for glucose.

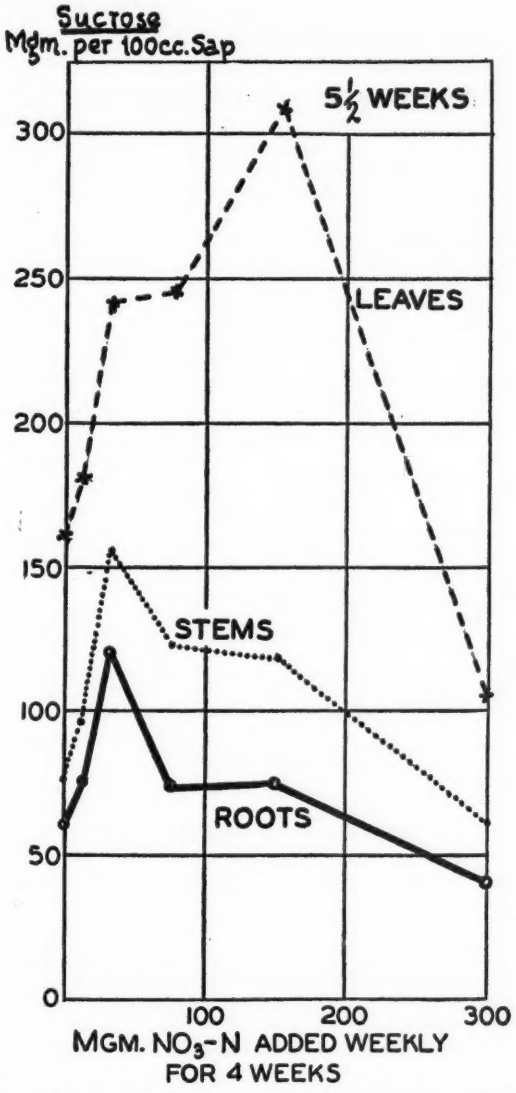

FIG. 3. SUCROSE CONTENT OF PLANT SAP AFTER NITRATE ADDITIONS HAVE CEASED

After 4 weeks the addition of nitrate was discontinued, so that the nitrogen supply for plants growing in the lower nitrate levels was soon exhausted

(fig. 1). The comparative concentrations of sugars under these conditions are shown in figure 3. Instead of a sharp decline in sugar, as in figure 2, the sugar concentration rose in the lower nitrate treatments, followed by a reduction. The change in type of curve from figure 2 to that of figure 3 was noticed in the various experiments to occur first for sucrose at approximately 5½ weeks, followed by glucose a week or more later. Factors that may have contributed to this change were at least two:

1. Since the nitrogen-hunger period in soybeans lasts until after the third week, the fixation process in plants harvested at 4 weeks was just getting underway. At 5 and 6 weeks

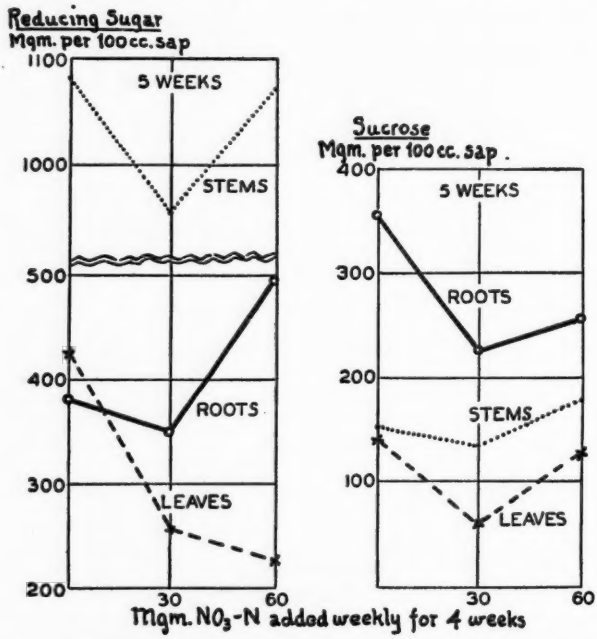

FIG. 4. EFFECT OF NITRATE ON SOLUBLE SUGAR IN SAP OF UNINOCULATED PLANTS

much larger quantities of nitrogen were being fixed, therefore the sugar level would tend to be lowered at these later harvests as more nitrogen was made available for protein formation.

2. In the plants that received low levels of nitrate, a photosynthetic stimulation, as has been noted, maintained and even raised the sugar level after the nitrate was depleted, in spite of the nitrogen supplied by fixation. In the higher nitrate applications enough nitrogen still remained to combine with the sugar formed and hold the free sugar at a lower level.

Since all plants in these experiments were inoculated, it is not clear whether the observed effects on the sugar content of the sap are due directly or indirectly to the combined nitrogen. It is possible that physiological effects of combined nitrogen on nodulation and nitrogen fixation are factors in regulat-

ing the carbohydrate level. To settle this question an experiment was made in which all plants receiving combined nitrogen (as NH_4NO_3) were uninoculated. Only the lower concentrations of combined nitrogen were used, since the occurrence of the maximum and minimum points in the curves was noted in previous experiments at concentrations less than 75 mgm. The data in figure 4 show that there is an initial reduction in sugar followed by a rise in plants which received all their nitrogen as NH_4NO_3. The results of these experiments would indicate that the effect of combined nitrogen on nodulation and nitrogen fixation is probably an indirect one, *viz.*, the presence of combined nitrogen lowers the sugar content, which affects the development of nodules. It appears that any direct physiological effect of combined nitrogen on nodulation is relegated to a secondary rôle.

Roux and Burgevin (7) have recently shown that the total quantity of nitrogen *fixed* increases with nitrate treatment within certain limits. This may well be explained by the increased carbohydrate made available through a photosynthetic stimulation by nitrate as observed in the present experiments. It has also been observed (10) that increased photosynthetic capacity increases nodulation, which is very probably a factor in increased nitrogen fixation.

SUMMARY

Data concerned with the carbohydrate metabolism of soybeans are presented which support a biochemical explanation of the effect of nitrate-nitrogen on nodule formation. In concentrations of nitrate *that do not prevent nodule formation* the addition of nitrate can effect the carbohydrate content in the following ways:—

A low nitrate concentration reduces the level of soluble sugars in the plant sap—probably through protein formation.

Intermediate concentrations of nitrate apparently stimulate photosynthesis, as evidenced by increased soluble sugars. It has been noted that this is associated with increased nodulation and nitrogen fixed (7, 10).

Higher nitrate concentrations again decrease the soluble sugar level because the abundant nitrogen source has made the photosynthetic capacity the limiting factor in protein formation. This second reduction of sugars is associated with decrease in number and size of nodules.

It appears that the carbohydrate level in the plant is correlated with nitrate-nitrogen concentration in much the same way as is nodule production and thus supports a chemical explanation of the phenomenon rather than a physiological interpretation.

REFERENCES

(1) Bryan, O. C. 1922 Effect of different reactions on the growth and nodule formation of soybeans. *Soil Sci.* 13: 279.

(2) Emmert, E. M. 1929 The determination of nitrate in green tomato and lettuce tissues. *Plant Physiol.* 4: 519–528.

(3) FRED, E. B., BALDWIN, I. L., AND MCCOY, E. 1932 Root nodule bacteria of leguminous plants. Monog. Univ. Wis. Studies Sci. 5.

(4) HOPKINS, E. W., AND FRED, E. B. 1933 Influence of various nitrogenous compounds and mannitol on nodule formation by clover. *Plant Physiol.* 8: 141–155.

(5) HOPKINS, E. W., WILSON, P. W., AND FRED, E. B. 1931 A method for the growth of leguminous plants under bacteriologically controlled conditions. *Jour. Amer. Soc. Agron.* 23: 32–40.

(6) MAZE, M. 1898 Le microbes des nodosites des Legumineuses. *Ann. Inst. Pasteur* 12: 1–35.

(7) ROUX, M. EUGENE, AND BURGEVIN, M. 1933 La fumure Azotee des Legumineuses. *Compt. Rend. Acad. Agr. France* 6: 186–190.

(8) STILES, H. R., PETERSON, W. H., AND FRED, E. B. 1926 A rapid method for the determination of sugar in bacterial cultures. *Jour. Bact.* 12: 427–439.

(9) STROWD, W. H. 1920 The relation of nitrates to nodule production. *Soil Sci.* 10: 343–356.

(10) WILSON, P. W., GEORGI, C. E., AND FRED, E. B. 1933 The effect of pCO_2 on nodule formation by clover in presence of combined nitrogen. Third Commission of Soil Microbiology and Soil Biochemistry of the Third International Soil Science Congress, Copenhagen, Denmark.

THE EFFECT OF LONG AND SHORT DAY AND SHADING ON NODULE DEVELOPMENT AND COMPOSITION OF THE SOYBEAN[1]

E. W. HOPKINS[2]

University of Chicago

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The fundamental problem of the necessity of light for the subsistence of plant life has received the attention of workers in many fields of science. In studies on root nodule formation by leguminous plants, however, light has not been considered in the same detail as have other factors which determine plant growth. Scattered observations upon nodule development under poor conditions of illumination have been made, but in only a few cases have data been recorded on plant response related to nodule development.

The seasonal variation in the intensity of light and length of day on nodule formation was considered by Leonard (15). Successive plantings were made of three varieties of soybeans in sand and soil at intervals of a month throughout almost a year. On those plants grown during the winter months when the days are short and the light intensity is low the number of nodules was at a minimum. Also, during the winter the plants produced the lowest dry weight. Decrease in dry weight was greater in plants of the same series than in those grown in soil. It was also found that removal of leaves greatly decreased the number of nodules which formed. It is suggested that these results are related to the decreased photosynthetic activity of the plants.

Studies of the photoperiodic response of plants grown under artificially shortened or increased day-lengths have included a number of leguminous plants. Comparison of nodule development under short and long daily periods of illumination have been reported by several workers. Rosenfels (23) grew Wisconsin Early Black soybeans in a rich and in a poor soil, exposing half of the plants to a 7-hour day and half to full day-length (October) with artificial lighting from 4–10 p.m. The results are expressed as the percentage of plants which developed nodules. A higher percentage of the short day than of the long day plants developed nodules, and within one length of day treatment the plants in the poor soil were better provided with nodules than were those in the rich soil. Carbohydrate analyses indicated the highest carbohydrate percentages to follow the best yields of nodules.

Tincker (30) exposed cultures of *Phaseolus multiflorus* to 5-hour, 10-hour,

[1] Contribution from the Hull Botanical Laboratory, University of Chicago.

[2] National Research Council Fellow.

and full day illumination. The plants grown in 5 and 10 hours of light possessed heavy, tuberized roots on which no nodules had formed, while plants grown in the full day did form nodules. The short day plants contained less fiber, but more crude protein and starch, than did the long day plants.

Wisconsin Early Black variety soybeans were grown by Eaton (5) in lengths of day of 3, 4, 5, 6, 8, 10 and 12 hours and full day length. The weight of nodules on these plants increased with the length of day, as did also the absolute amount of carbohydrates. In plants subjected to removal of leaves, the same relations were found to hold true.

Allam (1) attempting to determine the energy relation of nitrogen fixation in leguminous plants, gives results with peas and soybeans, a part of which were grown under short day conditions. The day length was not constant, but varied from 6 to 8 hours. Nodules on the short day plants were noticeably smaller than on the long day plants.

The application of nitrate to plant cultures is an important factor influencing nodule development, and will be dealt with in this paper. It will not be necessary to include the large number of references on the result of nitrogen fertilization on nodule development inasmuch as reviews are available (6). The papers considered here will be only those dealing with the composition of leguminous plants to which nitrates have been applied. Welton and Morris (32) grew Elton and Hamilton variety soybeans in poor, medium, and rich soils. The number of nodules decreased definitely with increasing fertility of the soil. Analyses of the lower two-thirds of the stems showed little difference between the percentage of available carbohydrates in plants from the poor and medium soils, though plants from the rich soil were lower in carbohydrates than were plants from poor or medium soil. Total carbohydrate percentages, including cellulose and lignin as well as the available carbohydrates, showed decreasing order with increasing soil fertility. The total, insoluble, and soluble nitrogen content of plant stems from the poor and rich soils were similar.

Thirteen periodic harvests were made by Rüffer (24) of Mammoth Yellow soybeans from three series: (*a*) nitrate, no nodules, (*b*) nitrate-nodule, (*c*) nodule. The nodules on the plants receiving nitrate were considerably smaller than those on the minus nitrogen plants. The application of nitrogen resulted in higher yields of plant material as fresh weight after the first few harvests, and as dry weight in later harvests. The total amount of nitrogen and the percentage of nitrogen in the tops and whole plant were higher in the nitrate-nodule series than in the nodule series until near the end of the experiment. Contrasted with the relation between nitrogen content in the three series is the content of sugar and starch. The nodule series was higher in percentage of sugars until the seventh harvest, and in starch until the last harvest. This work will be discussed in more detail later.

Reports of a few investigators had made it appear promising that a detailed examination of the nitrogen and carbohydrate constituents of leguminous plants growing under various conditions of light and nitrogen nutrition might

indicate some relationship between plant composition and nodule development. Also, various lines of indirect evidence appeared to suggest that such a relation did exist. Those treatments which tended to produce a high carbohydrate type of plant, as low nitrogen supply, increased CO_2 pressure, long day illumination, and bright light also are notably favorable for nodule development. Accordingly, experiments were planned to produce plants of varying carbohydrate composition from low to very high, with coincident inverse nitrogen composition.

The work of Garner and Allard (7) has shown that medium and late varieties of soybeans are definitely short day plants. Associated with the phenomenon of flowering in short day, the soybean accumulates considerable carbohydrate. Accumulation of nitrogen also occurs in such plants (18). These facts led to the selection of the soybean as the experimental plant. The following experiments will be reported here:

1. Long and short day experiments.
   Exp. 1. Plants grown under long and short photoperiods with varying levels of nitrogen nutrition.
   Exp. 2. Plants grown under long and short photoperiods without nitrogen.
2. Shading experiments.
   Exp. 3. Plants grown with and without shading with nitrate supplied.
   Exp. 4. Plants grown with and without shading without nitrogen.

METHODS

The plant used throughout the work was the Manchu variety of soybean.[3] Two- and three-gallon glazed coffee urn linings served as pots. These were filled with No. 3 quartz sand. For inoculation of the seed, one strain of *Rhizobium japonicum*, the Wisconsin strain number 504, was used. Inoculation was effected at the time of planting by suspending the bacterial growth from cultures about 2 weeks old in distilled water and soaking selected soybean seed in the bacterial suspension for about half an hour. In experiments 1 and 3 the seed was planted in beds of No. 3 quartz sand and seedlings were transplanted to the pots. In experiments 2 and 4 the seed was planted directly in the pots and selection made of the seedlings. No noticeable difference between the transplanted and directly planted seedlings was apparent.

For the first 2 weeks the plants were watered with tap water and after that time, with nutrient solutions. In experiment 1, the solution was supplied by the constant drip method of Shive and Stahl (26). In the other experiments water or nutrient solution was poured into the pots. In experiments 3 and 4, since the plants were out-of-doors, the pots were set in shallow granite-ware pans to prevent wide fluctuations in water supply. In all experiments, the pots were flushed out with about a gallon of water at weekly intervals. An attempt was made to maintain fairly constant pH conditions in each experi-

[3] The author is indebted to the A. L. Dickinson Seed Company of Chicago for the seed used.

ment. The pH in the several experiments was varied because the optimum pH for nitrate nutrition is different from that for nitrogen nutrition from nodules, as will be discussed later.

Two nutrient solutions were used. In experiment 1 and for the first crop of experiment 3 a solution used by Tiedjens and Robbins (29) was applied, and in the remaining experiments a Bryan's solution (3) was used. These were made up as minus nitrogen solutions and so used in experiments 2 and 4, whereas varying amounts of nitrate were added in experiments 1 and 3. The composition of these basic solutions expressed on the partial volume molecular basis is as follows:

Bryan's solution		*Tiedjens and Robbins' solution*	
K_2HPO_4	0.00143	KH_2PO_4	0.00633
$MgSO_4 \cdot 7H_2O$	0.00102	$MgSO_4 \cdot 7H_2O$	0.00237
$CaCl_2$	0.00225	$CaCl_2$	0.00146

The amounts of nitrate applied in each series will be given as each experiment is described.

In experiments 1 and 2, in which the plants received a long and a short photoperiod, the pots were placed on trucks. The "short day" plants received 7 hours of illumination, from 9 a.m. to 4 p.m. daily. For the long nights the trucks were wheeled into light-tight ventilated rooms. The temperature in these rooms was lower during the late afternoon than that in the greenhouse, but the night temperatures were nearly identical. The long days were obtained by supplementing the prevailing length of day from 5 to 9 p.m. with 1000-watt lamps. Shading in the out-of-door experiments—3 and 4—was produced by a gabled roof built over the table supporting the pots and covered with unbleached muslin. The muslin extended to the eaves of the roof, which were 4 feet above the table. The plants thus received full sunlight in the early morning and late afternoon, and were shaded through the middle of the day for a period of 5 to 7 hours.

The light transmission of the shade was determined by means of a MacBeth illuminometer, and the readings were taken with the test plate directed toward the sun. Under these conditions, the light measured under the shade was 16.9 per cent of the light outside on a bright sunny day. By the end of the experiment, the light transmission had been decreased by dust accumulation on the cloth to 11.7 per cent of full sunlight. The "sky" or diffuse light was reduced under the shade to 71.4 per cent of that outside.

Microchemical tests for starch and nitrate were made from time to time on the plants.

Plant harvests

The plants were subjected to the same light treatment on the night before harvesting as had been given throughout the experiment. Harvests were started in the morning, but in some cases the entire day was required to care for the plants in the different series.

Nodules were first picked from the roots, and the roots were severed near the sand line. The stems were picked free of leaves (including pulvini) and blossoms, and the upper inch was removed. In experiment 3, second crop, the lower stem fraction is that part from the base upward for 4 inches. Petioles were removed from the leaves and put into a heterogeneous fraction made up of stem tips, leaves of one third full size or smaller, buds, and fruit. This fraction was used for obtaining total plant weights and was not analyzed for carbohydrates. However, in order to obtain the nitrogen balance of the plants in experiments 2 and 4, nitrogen fractionations were made of the "Petiole, etc." fraction. The rachis bearing the trifoliate leaf was included with the leaf blades. The plant material was minced with a sharp knife, and that part used for carbohydrate and total nitrogen analyses was dried according to the procedure given by Link (16).

The samples for nitrogen fractionation were taken from the fresh material. In experiment 1 the tissue was extracted at once, but in the remaining experiments it was preserved by freezing and was maintained at -5 to -10°C. until analyzed a short time later (19).

Carbohydrate methods

The dried plant material was ground roughly in a hand drug mill, and the grinding completed in a ball mill.

Obvious steps in the analytical procedure will be omitted, and only those points given which are essential to the methods. The sugar determinations were made by a micro Shaffer-Hartmann method (27). In a large number of analyses this method has proved reliable and sufficiently accurate. It is also a rapid method. Sucrose was inverted with HCl (2).

Starch and dextrin were extracted from the sugar-free residue by boiling with water for about 5 minutes, and maintaining the solution at 80°C. for half an hour to an hour. The solution was cooled to 38°C. and 10 cc. of saliva added. Starch which had remained in the plant tissue during the heating and digesting treatment was removed by this procedure. The residue was always examined microscopically for the presence of starch and if none was found, the determination was continued. The maltose from the starch hydrolysis was converted to glucose by the method recommended by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists (Willaman et al. 34).

By "total carbohydrates" is meant the sum of total sugars and starch and dextrin. The figure represents the readily available carbohydrates in the plants.

Nitrogen methods

Preparation of soluble nitrogen extract. In experiments 1 and 3, 5 or 10 gm. of the minced and well-mixed plant tissue were ground and extracted with water

(18), and the extract was filtered through a pad of rag pulp on a Büchner funnel. In experiment 2, 10- to 20-gm. samples of the plant parts were used for nitrogen fractionation, and in experiment 4, 25 to 50 gm. of nodule material and 100 gm. of the other plant parts. It had been found (4) that extraction by boiling water removed the soluble nitrogen from plant material as efficiently as did grinding. The material from experiments 2 and 4 was extracted by boiling for 10 minutes, acetic acid was added to give a concentration of about 0.02 per cent, and boiling was continued for 5 minutes more. To facilitate removal of the soluble nitrogen, the nodule material was crushed before being extracted. After boiling, the material was washed and squeezed out a number of times and the extract filtered through a pad of rag pulp. Extracts prepared in this manner do not contain coagulable nitrogen, but only the "soluble nitrogen" of the plant material.

Nitrogen fractions determined. The total nitrogen of the extract was determined by the method recommended by Ranker (22). The ammonium nitrogen and the nitrate nitrogen were determined on duplicate aliquots by the method of Sessions and Shive (25). Amide nitrogen was split off by hydrolysis (19) and the resulting ammonia determined by aeration. The total nitrogen in the humin precipitate was determined for some of the early samples, but it constituted so small a part of the total soluble nitrogen that the precipitates from the later samples were discarded. The humin filtrate was treated according to the procedure of Osborne and Harris (21) for determination of the phosphotungstic acid precipitable nitrogen. This fraction will hereafter be referred to for convenience as "basic nitrogen" though only a small portion of the fraction is made up of the diamino acids. The filtrate was used for the α-amino nitrogen determination by the usual Van Slyke method.

All results are expressed as percentage nitrogen in the form given, not, for example, as NH_3 or NO_3.

The total nitrogen was determined on the dried samples by the Kjeldahl method modified to include the nitrogen of nitrates. "Insoluble nitrogen" is obtained by subtracting the figure for soluble nitrogen from that for total nitrogen. In experiments 1 and 3, the "inorganic nitrogen" determinations were made on the dried material, and include the nitrogen of both nitrate and ammonia.

In spite of the fact that small samples were taken in experiments 1 and 3 for the estimation of total extractable nitrogen, the amount of nitrogen found in duplicate aliquots checked satisfactorily in most cases. It is possible by making analyses on a semi-micro scale to perform nitrogen fractionations on 10 to 20 gm. of material as was done in experiment 2. Difficulty because of the small size of the sample was encountered only in the determination of amino nitrogen.

Insoluble ash was determined in the dried root samples, and data given for the roots are on the insoluble ash-free basis.

Plant growth

Experiment 1 (*Greenhouse*). The seed was planted March 9 and the harvest made April 25, 1932. Two series were set up in both long and short day, a very low nitrogen series, and a high nitrogen series. The nitrate, KNO_3 expressed as mgm. per liter of nitrogen added to the basic Tiedjens and Robbins' solution, is as follows: low nitrogen—2 mgm. 10th to 22nd day, 5 mgm. 23rd to 29th day, and 10 mgm. thereafter; high nitrogen—20 mgm. 10th to 20th day, 40 mgm. thereafter.

The pH of the nutrient solution was 5.2. The pH of drippings from the pots was 5.2 to 5.8.

There was no symptom of the usual nitrogen-hunger period[4] in any set except the low nitrogen, long day plants. A yellowing of the leaves began in this set about 4 weeks after planting. The low nitrogen, long day plants were hard and woody, and rather pale green. The high nitrogen, long day plants were green with large leaves, and were rapidly vegetative and somewhat taller than the low nitrogen set. Both sets of long day plants possessed flower buds at the time of harvest. The short day plants were somewhat paler in color than the long day high nitrogen plants and were considerably branched, but otherwise appeared like young plants. There were, of course, numerous blossoms on both the low and high nitrogen sets.

At no time were more than traces of nitrate nitrogen found in the long day, low nitrogen plants. The long day, high nitrogen plants gave strong tests for nitrates in all parts of the plant at most times. A slight accumulation of starch was noted in the low nitrogen set on the 34th day, but this had disappeared by the 47th day.

In the short day plants, nitrate accumulation was first noted on the 24th day in the high nitrogen set, and on the 34th day in the low nitrogen set. Between the 34th and 42nd days starch accumulation in the bases of stems started in both sets. At this time, large grains were present in the pith, xylem parenchyma, and phloem in what seemed to be similar amounts in both sets.

The treatments used considerably affected the size and distribution of nodules on the roots of plants in the various sets. On the long day, low nitrogen set, the nodules were very large; many of them were near the crown of the plant, though some were distributed over the fibrous roots. Nodules on the long day, high nitrogen set were considerably smaller, and were distributed over the fibrous roots more than were those of the low nitrogen plants. Nodules on the short day plants were considerably smaller than those on the long day

[4] Reference will be made from time to time in describing the growth of the plants to the "nitrogen-hunger period." This stage of development is that at which the nitrogen reserves of the seed have been exhausted. Fixation of nitrogen in the nodules has begun, but is not sufficient to supply the needs of the plant. Thus, if no fixed nitrogen is given the plant, nitrogen deficiency becomes evident. After a time, nitrogen fixation in the nodules proceeds more rapidly, and the plants suddenly become dark green, and produce rapid growth.

plants. The nodules of the high nitrogen plants were smaller than those of the low nitrogen ones.

Experiment 2 (*Greenhouse*). The seed was planted March 4, 1933, and three harvests were made. The first was of long and short day plants, on April 22; the second, of a few pots of long day plants, on April 27; and the third, of the remaining long and short day plants, on May 20. These plants were grown without the addition of fixed nitrogen, except what was obtained from the tap water. In this experiment the pH of the solution in the pots was maintained from 5.8 to 6.5. According to whether it was desired to raise or lower the pH in the pots, Bryan's nutrient solution was made up with K_2HPO_4 or KH_2PO_4.

About 4 weeks after planting, the long day plants began to show nitrogen starvation. The short day plants showed no definite symptoms of nitrogen deficiency. The first harvest was made when the plants were still in the nitrogen-hunger stage. At the second harvest (of long day plants only) nitrogen fixation by the nodules was evident from the dark green color of the plants and rapid new growth. The third harvest took place when the plants were beginning to produce fruit.

A few tests for nitrate were made, but with negative results. After 42 days, the short day plants had begun to accumulate starch in the bases of the stems. Until the end of the experiment, the masses of starch increased until the whole stem section appeared filled. No accumulation of starch was observed in the long day plants.

At the third harvest the long day plants were nearing maturity. The short day plants remained small. The stems were about 10 cm. long, and the numerous leaves which formed were very small and clustered close to the stem. Plate 1, figure 1, gives the appearance of the plants at the third harvest.

At the first harvest, the nodules on the two series of plants were similarly distributed on the roots, that is, clustered at the crown of the plant, with a few along the tap root, but those on the short day plants were considerably smaller. At the third harvest, it was evident that nodule formation had continued in the long day plants, with more nodules present along the tap root, and that the nodules had increased in size. The nodules on the short day plants apparently had increased very slightly in number, but were somewhat larger. Plate 1, figure 1, shows the difference between the two series.

Experiment 3 (*Out-of-doors*). The seed of the first crop was planted July 6, and the plants were harvested August 12, 1932. The second crop was planted August 10 and harvested September 22. In this experiment the plants were given considerable amounts of nitrate as KNO_3 in the first crop and as $Ca(NO_3)_2$ in the second. By "considerable" is meant an average of about 50 mgm. NO_3—N added to each pot daily after the plants were about 3 weeks old. All parts of the plant gave strong tests for nitrate. No starch was stored at the base of the stems. The pH of cultures set in pans is somewhat more difficult to control than when free drainage takes place, but the pH was maintained most of the time between 5.0 and 6.0.

The plants in the first crop suffered considerably from a nutritional disorder which produced wrinkled leaves (9). In later experiments this condition was prevented by using a nutrient solution with a higher Ca/K ratio. Since the plants responded in the same way as did those of the second crop which had no affected leaves and showed similar chemical composition to those of the second crop, the results are included.

There was no appearance of a nitrogen-hunger period in the plants of either crop.

Nodules on the shaded plants were smaller and were more scattered over the fibrous roots, rather than clustered largely around the crown as in the unshaded plants.

Experiment 4 (Out-of-doors). The seed was planted May 23, 1933, and two harvests were made, the first on July 1, and the second on July 27. A severe nitrogen-hunger stage began when the plants were about 3 weeks old. Growth appeared to stop entirely, and symptoms of some toxicity from the tap water began to be evident. High carbohydrate plants are extremely susceptible to the effect of manganese and boron, and it is possible that the toxicity was due to these elements or to others present in the tap water. A nitrate addition was given to combat this toxicity. The pH of the cultures had been maintained at 6.0 to 7.0 up to this time. The pH was then lowered to between 5.0 and 6.0, and 20 mgm. NO_3—N as $Ca(NO_3)_2$ was added to each pot. The plants responded at once to the nitrogen addition, were green for a few days, and then returned to the nitrogen-hunger stage. At this time, when the first harvest was made, there was no nitrate present in the plant, and starch had accumulated in the stems of the unshaded plants. The nitrogen deficiency of the shaded plants had been much less pronounced than that of the unshaded ones.

The second harvest was made after pods from the first blossoms had formed.

In both experiments 3 and 4 shading resulted in somewhat taller, more slender-stemmed plants with larger, greener, and possibly more numerous leaves. Nodules on the unshaded plants were clustered about the crown, with a few scattered over the root system. The shaded plants had somewhat smaller and less numerous nodules more diffusely scattered over the roots. Figure 2, plate 1, shows the appearance of the plants and roots from experiment 4 at the second harvest.

Various questions arise from the data given on the growth responses of the plants which it may be well to discuss before proceeding to the results of the chemical analyses of the plant parts.

One of these is the important question of the hydrogen-ion concentration at which the cultures should be grown. Much has been written concerning the optimum pH for nodule development on a number of host plants (6, p. 197). The majority of workers have concluded that a reaction near neutrality, of 6.0 to 7.0, is the most favorable for nodule development and yield of leguminous plants.

The importance of nearly neutral reaction was noted in the minus nitrogen

cultures, experiments 2 and 4. When the pH of the nutrient solution contained in the pot dropped below 6.0, the leaves of the plants showed chlorotic areas which again became green within a day or two if the pH was raised above 6.0. On the other hand, plants with a thoroughly healthy appearance were produced in the nitrate experiments, experiments 1 and 3, at a pH range below 6.0. Such plants produced large nodules, and nodule formation continued through the growth of the plant. Nitrate always had the effect of decreasing the size of the nodules and also of altering their distribution on the roots, as had been previously observed (11).

In comparisons of the benefit to leguminous plants of nitrogen fixed in the nodule with nitrogen from combined sources, the plant cultures of both series have been kept at the reaction which is most favorable for nodule formation, near neutrality. That a high pH is not the most favorable reaction for obtaining maximum plant yields with nitrates has been shown for a number of plants by Tiedjens and Robbins (29) and by others. On the other hand, ammonium salts produce the highest plant yields at a reaction above 7.0. Thus the nitrate series grown near neutrality are placed at a disadvantage.

Weber (31) supplied Victoria peas with nitrate in cultures in which the reaction ranged from 6.6 to 7.6 and compared the yield and total amount of nitrogen in the plants with nodule cultures given no nitrate. Although at first, presumably until the end of the nitrogen-hunger period in the nodule series, the nitrate series yielded the greater weight of plants and total amount of nitrogen in plant yield, the nodule series were later superior. Weber draws the conclusion that leguminous plants produce greater yields and contain more nitrogen when the nodule, rather than nitrate, serves as the source of nitrogen. A contrary result is usually obtained, however, when NH_4NO_3 is used as the source of nitrogen, as in the work of Allam (1). The data of Rüffer, particularly, show generally an increased yield of fresh and dry weight of tops and total amount of nitrogen in the plants grown with nitrogen supplied as NH_4NO_3 compared with those depending on nitrogen from the nodule. In the plants of the nodule series, however, the percentage of nitrogen is usually higher late in the experiments when nitrogen fixation is proceeding at a rapid rate. Even late in the experiments, the yield of plant material still continues to be higher in the NH_4NO_3 series. The plant cultures of Rüffer varied in pH from 6.7 to 7.3. Although this range is not the optimum for the utilization of nitrate, it approaches the optimum for ammonium salts. Thus the plants in such cultures are able to feed largely on ammonium nitrogen.

It thus appears that the plants of Rüffer supplied with NH_4NO_3 produced generally larger plants containing more nitrogen than did nodule plants because the plant cultures were maintained at a pH which was favorable for the utilization of the nitrogen of the ammonium ion. The NH_4NO_3 plants did not show nitrogen starvation. Weber, using $NaNO_3$ at a pH unfavorable for its utilization, obtained greater yields from nodule plants. Although sufficient nitrogen was present in the $NaNO_3$ series, the plants showed the effect of nitrogen

starvation. The plants in experiments 1 and 3 maintained at pH of 5.0 to 6.0 and supplied with nitrate showed no nitrogen deficiency.

RESULTS

Some means of expressing the extent of nodule development in the various series was desired. The number or weight of nodules per plant would not give a fair notion of the relation between nodule development and plant growth inasmuch as both large and small plants were to be compared. An index was desired which would take into consideration the vegetative growth of the plant, and which also could be expressed on a percentage basis, as are the chemical results. The index selected as being most suitable for this work was that of the weight of nodules expressed as percentage of the fresh weight of the entire plant. This figure will be referred to hereafter for convenience as "the percentage of nodules."

In the short and long day experiments, the percentage of nodules was appreciably affected by the treatments used.

Experiment 1. Plus nitrate.	*Low N*	*High N*
Short day	2.27	1.15
Long day	3.77	2.34

Experiment 2. Minus nitrate.	*1st harvest*	*3rd harvest*
Short day	1.56	2.68
Long day	5.67	5.51

Short day conditions were less favorable than long day conditions for nodule development. Rosenfels (23) reports better nodule development on soybeans grown in short than in long day. The foregoing results show that within one day length, the percentage of nodules was decreased by the high nitrogen treatment.

The effect of shading on the percentage of nodules may be seen from the following figures:

Experiment 3. Plus nitrate.	*1st crop*	*2nd crop*
Shaded	1.43	2.21
Unshaded	3.06	3.22

Experiment 4. Minus nitrate.	*1st harvest*	*2nd harvest*
Shaded	5.17	6.56
Unshaded	5.75	7.61

There is a difference in nodule development in favor of the unshaded plants, though it may be seen that the difference between shaded and unshaded plants is smaller when no nitrate is given.

The analytical data for the experiments in which the plants received long and short photoperiods are given in figures 1 and 2, and table 1.

Figure 1 shows the results obtained from the plants of experiment 1 in which the plants received long and short photoperiods and very low and high nitrogen nutrient. The stems of the long day plants were higher in percentage moisture. Very great accumulation of carbohydrates and nitrogen had occurred in the short day plants. Total, total extractable, and inorganic nitrogen are definitely higher in short day than in long day plants. The greater nitrate

TABLE 1

Nitrogen composition, wet basis, of soybeans grown in long and short day, minus nitrogen—Experiment 2

Plants of first harvest 49 days old, of third harvest 77 days old

		AMMONIUM N	NITRATE N	AMIDE N	α-AMINO N	BASIC N	SOLUBLE N	INSOLUBLE N	TOTAL N
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roots:									
1st harvest	Short day	.0032	.0038	.0029	.0107	.....	.0342	.0898	0.124
	Long day	.0040	.0004	.0026	.0007	.....	.0238	.1132	0.137
3rd harvest	Short day	.0037	.0005	.0037	None	.0224	.0213	.0817	0.103
	Long day	.0034	.0005	.0042	None	.0177	.0395	.1185	0.158
Stems:									
1st harvest	Short day	.0077	.0103	.0077	.0274	.....	.4580	.2080	0.666
	Long day	.0039	.0017	.0038	.0073	.....	.0584	.1176	0.176
3rd harvest	Short day	.0110	.0022	.0742	.0517	.0938	.4170	.2070	0.624
	Long day	.0017	.0015	.0164	.0144	.0216	.0995	.1125	0.212
Leaves:									
1st harvest	Short day	.0040	.0015	.0092	.0023	.....	.1983	.7157	0.914
	Long day	.0026	.0005	.0042	.0219	.....	.1275	.6365	0.764
3rd harvest	Short day	.0046	.0004	.0108	.0353	.0600	.1550	.7120	0.867
	Long day	.0022	.0007	.0127	.0170	.0449	.1290	.6550	0.784
Petioles, etc.:									
3rd harvest	Short day	.0122	.0007	.0341	.0383	.0534	.2370	.3170	0.554
	Long day	.0039	.0002	.0096	.0118	.0327	.0870	.2150	0.302
Nodules:									
1st harvest	Long day	.0103	None	.0086	.0314	.....	.2935	.6635	0.957
3rd harvest	Short day	.0258	None	.0497	.0410	.2660	.5200	.7120	1.232
	Long day	.0137	.0047	.0274	.0531	.1801	.4445	.5735	1.018

additions in the high nitrogen series are seen to have resulted in a decrease in carbohydrate and an increase in nitrogen percentages in the long day plants. In the short day plants, the accumulation of nitrogen was somewhat increased by the higher nitrate level in the nutrient.

Soybean plants receiving a short photoperiod and given no fixed nitrogen accumulate large amounts of carbohydrates (fig. 2, exp. 2). Although the carbohydrate percentages was very high at the time of the first harvest, the total carbohydrates in the stems of the short day plants had more than doubled

at the time of the third harvest. At both harvests, the roots of the long day plants contained higher percentages of carbohydrates than did those of short day plants.

The nodules of the long day plants contain high percentages of carbohydrates in relation to the other parts of the plant. The percentages of carbohydrates decreased from the first to the third harvest.

Table 1 presents the nitrogen results from experiment 2. In the roots, total and insoluble nitrogen are higher in the long day plants at both the first and third harvests.

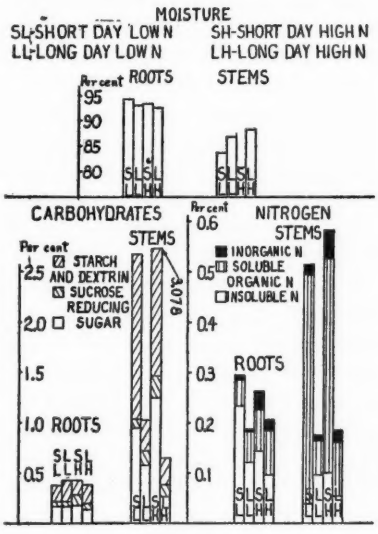


FIG. 1

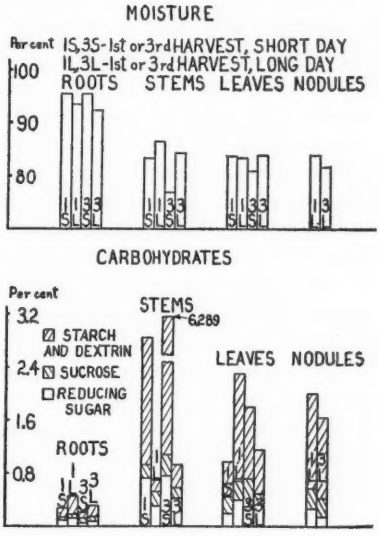


FIG. 2

FIG. 1. EXPERIMENT 1. CARBOHYDRATE AND NITROGEN COMPOSITION, WET BASIS, OF SOYBEANS GROWN IN LONG AND SHORT DAY. PLUS NITRATE. PLANTS 47 DAYS OLD

FIG. 2. EXPERIMENT 2. CARBOHYDRATE COMPOSITION, WET BASIS, OF SOYBEANS GROWN IN LONG AND SHORT DAY. MINUS NITROGEN. PLANTS OF FIRST HARVEST 49 DAYS OLD, OF THIRD HARVEST 77 DAYS OLD

The stems of the short day plants were higher in all forms of nitrogen at both harvests. The leaves of the short day plants were higher at both harvests in ammonium, soluble, insoluble, and total nitrogen. The petioles of the short day plants were higher in all forms of nitrogen than the corresponding parts of long day plants. Nodules from the short day plants contained a higher percentage of all forms of nitrogen except nitrate and α-amino nitrogen. If these results are computed as percentages of the total nitrogen, however, the differences between those for the short and the long day plants are not significant.

A comparison of the changes which occurred in the plants from the first to

the third harvest, will show that in the short day plants there was a percentage increase in the simpler forms of nitrogen: ammonium, amide, and α-amino. The soluble and total nitrogen percentages decreased. In order to determine whether this decrease in the soluble and total nitrogen had been due to a break-down to simpler compounds, as was indicated by the increased percentage of simpler forms of nitrogen, the various nitrogen fractions were computed as percentages of the soluble nitrogen. It was found that the amide and α-amino nitrogen made up a higher percentage of the soluble nitrogen at the time of the third harvest than at the first. Thus it appears that as short day plants grow older, the nitrogen constituents actually become more simple rather than being built into more complex forms. It is of interest that about two-thirds of the total nitrogen in the stems is in the soluble form.

In the long day plants, while the amide and α-amino nitrogen increased in percentage from the first to the third harvest, the soluble and total nitrogen also increased. The difference, it will be noted, between this result and that of the short day plants is the increase in soluble and total nitrogen. When nitrogen is applied to low nitrogen plants, the changes which take place in the nitrogen composition of the plants are similar to those seen in the long day plants (20). In this case, the plants received their nitrogen from the nodules.

The stems of the short day plants, and nodules of both long and short day plants at the third harvest were analyzed for α-amino nitrogen before and after the phosphotungstic acid precipitation. The short day stems apparently contain none of the basic amino acids, whereas about half of the amino nitrogen in the nodules is present in that form.

As has been indicated, the second harvest did not include any short day plants. Five days after the first harvest, the long day plants showed definite evidence of nitrogen fixation by the nodules. It was thought that a nitrogen fractionation performed at this time might reveal a change in the composition from that existing at the time of the first harvest. Several pots of plants were harvested, and the plants analyzed. Differences were found only in the total, insoluble, and soluble nitrogen content. All forms showed a considerable increase at the time of the second harvest.

The total amount of nitrogen in the plants at the three harvests was computed. The following figures give the milligrams of nitrogen in 10 plants.

	1st harvest	*2nd harvest*	*3rd harvest*
Short day	90.8	.....	179.8
Long day	248.8	406.3	867.6

Nitrogen in 10 seeds—83 mgm.

It is noteworthy that a very considerable amount of nitrogen had been fixed by the long day plants at the time of the first harvest, though, to all appearances, the plants were suffering greatly from lack of nitrogen. Weber (31) has obtained similar results. During the 5 days between the first and second harvests, over 150 mgm. of nitrogen were fixed by 10 plants. At the time of

the first harvest, the short day plants had fixed hardly any nitrogen, but had made a gain of about 90 mgm. at the third harvest.

The changes in nitrogen distribution in the nodules of the long day plants at the three harvests are presented in table 2. The total and insoluble nitrogen reached a maximum percentage at the time of the second harvest. Expressed as percentages of the total nitrogen the insoluble nitrogen decreased after the first harvest. The break-down of the complex nitrogen compounds in the nodule to simpler forms began soon after the first harvest. As the insoluble nitrogen decreased, the soluble nitrogen increased.

The results from the shading experiments are given in figures 3 and 4 and table 3. When nitrate is supplied to the plants, the carbohydrate percentages are low (fig. 3, exp. 3). Generally, all parts of the unshaded plants contained somewhat higher percentages of sugars and total carbohydrates than did the shaded ones. The nodules on either type of plant were higher in total carbohydrates than any other plant parts analyzed.

Inorganic, total extractable, and total nitrogen percentages were generally higher in the shaded plants than in the unshaded ones.

In the minus nitrogen experiment (fig. 4, exp. 4), the carbohydrate percentages were generally higher in unshaded plants at both the first and second harvest. Both shaded and unshaded plants showed a general decrease in starch and dextrin and total carbohydrates from the first to the second harvest. The decrease in carbohydrates in the nodules is of especial interest. The nitrogen data are presented in table 3. The nitrogen percentages in the stems, leaves, and petioles were higher in the shaded plants at both harvests. For the nodules, the results are irregular.

During the period between the first and second harvests, there was, in general, an increase in the percentage of all forms of nitrogen in both shaded and unshaded plants. The greatest increases took place in amide and α-amino, basic and soluble nitrogen. When the nitrogen results were calculated as percentages of soluble nitrogen, the change in proportion of the various nitrogen constituents during the interval between the first and second harvests were insignificant in any but a few plant fractions. At the second harvest, amide nitrogen made up a greater part of the soluble nitrogen in the stems than at the first. In the nodules, the basic nitrogen was relatively higher at the second harvest, while in the rest of the plant this fraction had decreased. If the results for basic, soluble, and insoluble nitrogen in the nodules are computed as percentages of the total nitrogen, it will be found that the basic and soluble nitrogen have increased. At the same time, there had been a decrease in the insoluble nitrogen. It is apparent that a proteolytic process was under way in the nodules, and the nitrogen made available was presumably being used for seed production (*see* table 2). Nodule material examined from time to time had indicated that decomposition at the center of the nodules was taking place. In other parts of the plants, the soluble nitrogen increased, and the insoluble nitrogen decreased somewhat in relation to the total nitrogen. These

TABLE 2

Nitrogen fractions in long day nodules—Experiment 2

Expressed as per cent, wet basis, and as per cent total nitrogen

	1ST HARVEST, 49 DAYS		2ND HARVEST, 54 DAYS		3RD HARVEST, 77 DAYS	
	Wet basis	Total N	Wet basis	Total N	Wet basis	Total N
Amide N	0.0086	1.0	0.0122	1.0	0.0274	2.7
α-amino N	0.0314	3.3	0.0393	3.3	0.0531	5.2
Soluble N	0.2935	30.7	0.3855	32.3	0.4445	43.7
Insoluble N	0.6635	69.3	0.8065	67.7	0.5735	56.3
Total N	0.9570	100.0	1.1920	100.0	1.0180	100.0

TABLE 3

Nitrogen composition, wet basis, of shaded and unshaded soybeans, minus nitrogen—Experiment 4

Plants of first harvest 39 days old, of second harvest 77 days old

		AMMONIUM N	NITRATE N	AMIDE N	α-AMINO N	BASIC N	SOLUBLE N	INSOLUBLE N	TOTAL N
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roots:									
1st harvest	Shaded	.0021	.0003	.0032	.0076	.0085	.0197	.0863	0.106
	Unshaded	.0027	.0006	.0042	.0095	.0102	.0223	.0947	0.117
2nd harvest	Shaded	.0033	.0012	.0073	.0301	.0140	.0462	.1368	0.183
	Unshaded	.0026	.0006	.0052	.0119	.0096	.0324	.1136	0.146
Stems:									
1st harvest	Shaded	.0023	.0004	.0066	.0115	.0206	.0490	.1360	0.185
	Unshaded	.0016	.0006	.0048	.0130	.0163	.0410	.1270	0.168
2nd harvest	Shaded	.0071	.0020	.0259	.0329	.0297	.1212	.2298	0.351
	Unshaded	.0062	.0010	.0214	.0238	.0266	.1005	.2095	0.310
Leaves:									
1st harvest	Shaded	.0041	.0014	.0084	.0244	.0487	.0985	.5125	0.611
	Unshaded	.0031	.0006	.0065	.0222	.0400	.0860	.4170	0.503
2nd harvest	Shaded	.0065	.0019	.0197	.0418	.0733	.1820	.9410	1.123
	Unshaded	.0044	.0016	.0110	.0395	.0770	.1640	.8940	1.058
Petioles, etc.:									
1st harvest	Shaded	.0028	.0007	.0065	.0161	.0252	.0610	.2190	0.280
	Unshaded	.0021	.0012	.0060	.0177	.0254	.0547	.1883	0.243
2nd harvest	Shaded	.0077	.0029	.0233	.0332	.0461	.1461	.2919	0.438
	Unshaded	.0063	.0013	.0191	.0287	.0466	.1245	.2645	0.389
Nodules:									
1st harvest	Shaded	.0132	.0001	.0326	.0422	.1462	.2980	.5720	0.870
	Unshaded	.0172	.0001	.0284	.0426	.1030	.2310	.4650	0.696
2nd harvest	Shaded	.0191	.0042	.0580	.0872	.2954	.5560	.5230	1.079
	Unshaded	.0170	.0044	.0818	.0840	.2690	.5010	.5810	1.082

changes were relatively small compared to those taking place in the nodules. The number of milligrams of nitrogen in 10 plants was as follows:

	1st harvest	*2nd harvest*
Shaded	322.2	2,090
Unshaded	261.9	1,660

Nitrogen in 10 seeds—83 mgm.

Thus, the amount of nitrogen fixed by the shaded plants considerably exceeded that fixed by plants in full sunlight in spite of the fact that the nodules on the

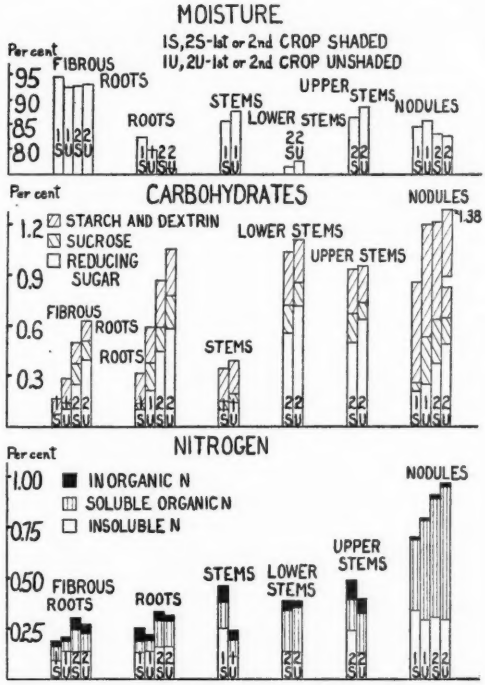


FIG. 3. EXPERIMENT 3. CARBOHYDRATE AND NITROGEN COMPOSITION, WET BASIS, OF SHADED AND UNSHADED SOYBEANS. PLUS NITRATE. PLANTS OF FIRST CROP 37 DAYS OLD, OF SECOND CROP 43 DAYS OLD

latter made up a greater part of the plant weight. As was described in the section on methods, the shaded plants received direct sunlight in early morning and late afternoon. They were thus shaded during the hottest part of the day. Garner and Allard (8) have found that darkening during the middle of the day had little effect on flowering. Photosynthetic activity appears to be less in plants of temperate and some of southern origin during the hours near noon (17, 12, 13). Possibly the method of shading produced a light intensity more favorable for photosynthesis than that of full sunlight. It is certain that

the shading treatment did not seriously inhibit the growth, and, although the plants differed in type from those grown in full light, they were by no means lacking in sufficient carbohydrate as is evidenced by the production of flower and seed. Temperature readings were taken of the sand in the pots of the shaded and unshaded series. In the morning, the sand in pots in direct sunlight was several degrees warmer than that in the shade. Air temperature under the shade and in direct sun differed by 1°C.

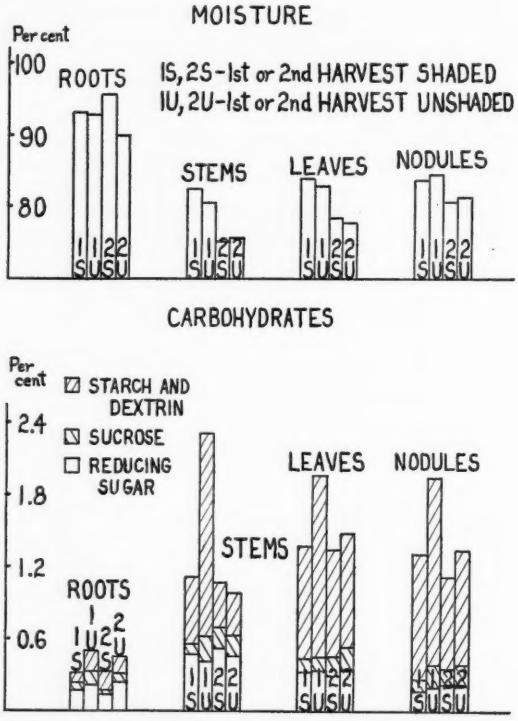


FIG. 4. EXPERIMENT 4. CARBOHYDRATE COMPOSITION, WET BASIS, OF SHADED AND UNSHADED SOYBEANS. MINUS NITROGEN. PLANTS OF FIRST HARVEST 39 DAYS OLD, OF SECOND HARVEST 77 DAYS OLD

DISCUSSION

A number of workers have indicated that conditions which favor carbohydrate synthesis are also those which favor nodule development. Whiting and Schoonover (33) determined that the rate of nitrogen fixation by cowpeas is related to the available carbohydrates. Leonard (15) has expressed a similar view. Wilson, Fred, and Salmon (35) found that increasing the concentration of CO_2 in the atmosphere greatly stimulated growth of red clover plants and resulted in an increase of the amount of nitrogen fixed per plant.

Much significance has been attached by a number of workers to the fact that the nodules of leguminous plants contain quantities of starch (6). The assumption was made that this starch is used by the nodule bacteria as a source of energy for the process of nitrogen fixation. Microchemical examinations of nodules from the plants of the various series had shown the presence of definitely less starch in the nodules of plants grown in shade or under short day conditions. The differences observed microscopically were far greater than those shown by chemical analysis (figs. 1, 2, 3, and 4). The analyses do show a decrease in starch as the plants became older. Numerous observations on this fact have been presented before (6). Since other parts of the plants show a similar decrease, however, the suggestion is offered that the nodules, with the rest of the plant, had drawn upon the stored starch.

The plants of experiment 4 (fig. 4) show a general decrease in the percentage of carbohydrates from the first to the second harvests. In experiment 2 (fig. 2), with the exception of the stems, the carbohydrates in the long day plants decreased from the first to the third harvest. Up to the first harvest in experiment 4 during the nitrogen-hunger period, the carbohydrates accumulated. After nitrogen fixation had proceeded for some time, the carbohydrates generally decreased. Rüffer (24), making harvests at 3-day intervals, observed an increase of carbohydrates in his plants as the hunger stage came on, and that after nitrogen fixation had begun, the percentage carbohydrates was lower in inoculated plants than in plants receiving fixed nitrogen. Rüffer suggests that up to and during the hunger stage, the accumulation of carbohydrates is due to stimulating action of the nodule bacteria on the assimilative power of the plant. After nitrogen fixation has started, however, the bacteria require so much carbohydrate as a source of energy for the fixation of nitrogen that the carbohydrate percentage is depressed below that in plants receiving fixed nitrogen. However, the energy requirements of the nodule bacteria for fixation of nitrogen is a very uncertain question (6, 36), and for the present at least, it seems wiser to attribute the change in carbohydrates of legumes after the nitrogen-hunger stage to causes other than increased consumption of carbohydrates by the nodule bacteria. An explanation of the results of Rüffer and those presented here is obvious in terms of known plant response.

Coincident with the drop in carbohydrates, Rüffer's figures show an increase in the total amount of nitrogen in the plant. The data in tables 1 and 3 indicate a considerable increase in percentage of all forms of nitrogen. These changes find an exact parallel in non-leguminous plants which are grown without nitrogen for a time and are then given fixed nitrogen (14, 20). Increasing nitrate additions to soybeans decreased the amount of sugar in the sap (28). As may be seen from figure 1, the long day, high nitrogen plants are lower in all forms of carbohydrate than are the low nitrogen series.

The conclusion which seems most reasonable from the foregoing data is that inoculated legumes grown without nitrogen decrease sharply in percentage of carbohydrates after the nitrogen-hunger period, because the carbohydrate supply is drawn upon for protein synthesis. If the nitrogen in the nodule is

fixed as ammonia or nitrate, combination of the nitrogen with carbohydrates must take place if the nitrogen is to become available to the plant. Or if nitrogen is fixed as an organic compound, carbohydrate would serve as the source of carbon.

SUMMARY

There is, generally, in the four experiments a higher percentage of sugars and starch in the roots of the plants which yielded the larger percentage of nodules. The only exception is the short day, high nitrogen set in experiment 1. Even in experiment 2, where the short day stems accumulated large amounts of sugars and starch, the roots of the long day plants contained a higher percentage of carbohydrates.

A number of lines of indirect evidence, particularly the fact that those treatments which decrease photosynthesis or reduce the carbohydrate supply of the plant are unfavorable for nodule development, had led the writer to interpret previous results (10, 11) as indicating that the carbohydrate supply of the plant was a determining factor in nodule development. The present work suggests that, with the exception of short day-length treatment, those treatments favoring nodule development generally produce plants high in carbohydrates. Of two series in long day; whether shaded or given low or high nitrate treatment, the series developing the higher percentage of nodules in relation to the whole plant weight was also usually higher in carbohydrates. The roots were more consistent in this relation than any other plant part.

The nitrogen data permit a more general statement than do the carbohydrate data. Comparing two treatments, the one which resulted in the higher soluble nitrogen percentage in all plant parts produced the lower percentage of nodules in relation to the whole plant weight. The percentages of amide and α-amino nitrogen, as well as total nitrogen in most instances, varied with the soluble nitrogen.

That those treatments favoring nodule development may not be most favorable for the highest fixation of nitrogen is shown by the results of experiment 4. Although the unshaded plants exceeded the shaded ones at both harvests in percentage nodules in relation to the whole plant weight, the shaded plants fixed considerably more nitrogen. No data are at hand which will explain this result.

CONCLUSIONS

Soybean plants were grown in long and short days, about 16 and 7 hours respectively, with a high and low nitrate series in one experiment, and minus nitrate in another. Shaded and full light experiments, plus and minus nitrate, were performed out-of-doors. Nodule formation was observed, and the plants were analyzed for carbohydrate and nitrogen fractions.

The short day-length plants accumulated much starch and were also high in nitrogen, in both the plus and minus nitrate experiments. In the minus nitrate experiment, carbohydrate accumulation increased in the short day-

length plants as the plants became older. The nitrogen was present in more simple compounds at the second harvest than at the first.

The plants receiving long day treatment were lower in all forms of nitrogen and in carbohydrates, particularly in the stems, than the short day-length plants. In the minus nitrate experiment, a first harvest was made at the time of the nitrogen-hunger stage, and another when the plants were in bloom. The carbohydrate percentages of roots, leaves, and nodules decreased, and all forms of nitrogen greatly increased in all plant parts as the plants grew older.

The shaded plants in both the plus and minus nitrate experiments were generally lower than the unshaded ones in carbohydrates and higher in all forms of nitrogen. In the minus nitrate experiment, both shaded and unshaded plants decreased in percentage carbohydrates from the first harvest, made during the nitrogen-hunger stage, to the second, made when the plants were producing fruit. All forms of nitrogen increased in both series from the first to the second harvest.

The weight of nodules expressed as percentage of the whole plant weight was decreased by high nitrate, by short day, and by shading. In general, those treatments which result in the accumulation of soluble nitrogen do not favor nodule development. Except in short day plants, those conditions which produce high carbohydrate plants are favorable to nodule development.

REFERENCES

(1) Allam, F. 1931 Vom Energieverbrauch der Knöllchenbakterien bei der Bindung des Luftstickstoffs. *Ztschr. Pflanzenernähr. u. Düngung* (A) 20: 270–301.

(2) Association of Official Agricultural Chemists 1925 Methods of Analysis, ed. 2. Washington.

(3) Bryan, O. C. 1922 Effect of different reactions on the growth and nodule formation of soybeans. *Soil Sci.* 13: 271–287.

(4) Davidson, O. W., Clark, H. E., and Shive, J. W. 1934 The preparation of aqueous extracts of soluble nitrogen from plant tissues. *Plant Physiol.* 9: 817–822.

(5) Eaton, S. V. 1931 Effects of variations in day-length and clipping of plants on nodule development and growth of soybean. *Bot. Gaz.* 91: 113–143.

(6) Fred, E. B., Baldwin, I. L., and McCoy, E. 1932 Root Nodule Bacteria of Leguminous Plants. Monog., Univ. Wis. Studies Sci. 5.

(7) Garner, W. W., and Allard, H. A. 1919–1920 Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *Jour. Agr. Res.* 18: 553–606.

(8) Garner, W. W., and Allard, H. A. 1931 Effect of abnormally long and short alterations of light and darkness on growth and development of plants. *Jour. Agr. Res.* 42: 629–651.

(9) Hopkins, E. W. 1933 Leaf-wrinkle, a nutritional disorder of soybean. *Plant Physiol.* 8: 333–336.

(10) Hopkins, E. W., and Fred, E. B. 1933 Influence of various nitrogenous compounds and mannitol on nodule formation by clover. *Plant Physiol.* 8: 141–155.

(11) Hopkins, E. W., Wilson, P. W., and Peterson, W. H. 1932 Influence of potassium nitrate on nodule formation and nitrogen fixation by clover. *Plant Physiol.* 7: 597–611.

(12) Kostytschew, S., and Berg, V. 1930 Untersuchungen ueber den Tagesverlauf der Photosynthese in Transkaukasien (Küstenregion des Schwarzen Meeres). *Planta* 11: 144–159.

(13) KOSTYTSCHEW, S., AND KARDO-SYSSOIEWA, H. 1930 Untersuchungen ueber den Tagesverlauf der Photosynthese in Zentralasien. *Planta* 11: 117–143.

(14) KRAUS, E. J., AND KRAYBILL, H. R. 1918 Vegetation and reproduction with special reference to the tomato. Oreg. Agr. Exp. Sta. Bul. 149.

(15) LEONARD, L. T. 1926 A preliminary note on the formation of photosynthetic carbohydrate to nodule formation on soybeans. *Jour. Amer. Soc. Agron.* 18: 1012–1016.

(16) LINK, K. P. 1925 Effects of the method of desiccation on the carbohydrates of plant tissues. *Jour. Amer. Chem. Soc.* 47: 470–476.

(17) LUBIMENKO, M. V. 1928 La biologie de la photosynthese. *Rev. Gén. Bot.* 40: 486–512.

(18) NIGHTINGALE, G. T. 1927 The chemical composition of plants in relation to photoperiodic changes. Wis. Agr. Exp. Sta. Res. Bul. 74.

(19) NIGHTINGALE, G. T., ROBBINS, W. R., AND SCHERMERHORN, L. G. 1927 Freezing as a method of preserving plant tissue for the determination of nitrogenous fractions. N. J. Agr. Exp. Sta. Bul. 448.

(20) NIGHTINGALE, G. T., SCHERMERHORN, L. G., AND ROBBINS, W. R. 1928 The growth status of the tomato as correlated with organic nitrogen in roots, stems, and leaves. N. J. Agr. Exp. Sta. Bul. 461.

(21) OSBORNE, T. B., AND HARRIS, I. F. 1903 Nitrogen in protein bodies. *Jour. Amer. Chem. Soc.* 25: 323–353.

(22) RANKER, E. R. 1926 Determination of total nitrogen, nitrate-nitrogen, and total nitrogen not including nitrate-nitrogen: Further observations on a modification of the official salicyclic-thiosulfate method. *Ann. Missouri Bot. Gard.* 13: 391–424.

(23) ROSENFELS, R. 1927 The relation of length of day to growth expression and chemical constitution in the soybean. B. S. Thesis, Univ. Wis.

(24) RÜFFER, E. 1932 Forschungen zum Kohlenhydratumsatz bei knöllchentragenden und knöllchenfreien Sojabohnen. *Ztschr. Pflanzenernähr. u. Düngung* (A) 24: 129–167.

(25) SESSIONS, A. C., AND SHIVE, J. W. 1928 A method for the determination of inorganic nitrogen in plant extracts. *Plant Physiol.* 3: 499–511.

(26) SHIVE, J. W., AND STAHL, A. L. 1927 Constant rates of continuous solution renewal for plants in water culture. *Bot. Gaz.* 84: 317–323.

(27) STILES, H. R., PETERSON, W. H., AND FRED, E. B. 1926 A rapid method for the determination of sugar in bacterial cultures. *Jour. Bact.* 12: 427–439.

(28) STROWD, W. H. 1920 The relation of nitrates to nodule production. *Soil Sci.* 10: 343–356.

(29) TIEDJENS, V. A., AND ROBBINS, W. R. 1931 The use of ammonia and nitrate nitrogen by certain crop plants. N. J. Agr. Exp. Sta. Bul. 526.

(30) TINCKER, M. A. H. 1928 The effect of length of day upon the growth and chemical composition of the tissues of certain economic plants. *Ann. Bot.* 42: 101–140.

(31) WEBER, E. 1930–1931 Salpeterdüngung als Beeinträchtigung des Stickstoffsammlung durch Leguminosen. *Centrlbl. Bakt.* (II) 82: 353–379.

(32) WELTON, F. A., AND MORRIS, V. H. 1930 Effect of fertility on the carbohydrate-nitrogen relation in the soybean. *Plant Physiol.* 5: 607–612.

(33) WHITING, A. L., AND SCHOONOVER, W. R. 1920 Nitrogen fixation by cowpeas and nodule bacteria. *Soil Sci.* 10: 411–420.

(34) WILLAMAN, J. J., ET AL. 1927 The determination of polysaccharides. *Plant. Physiol.* 2: 91–97.

(35) WILSON, P. W., FRED, E. B., AND SALMON, M. R. 1933 Relation between carbon dioxide and elemental nitrogen assimilation in leguminous plants. *Soil Sci.* 35: 145–165.

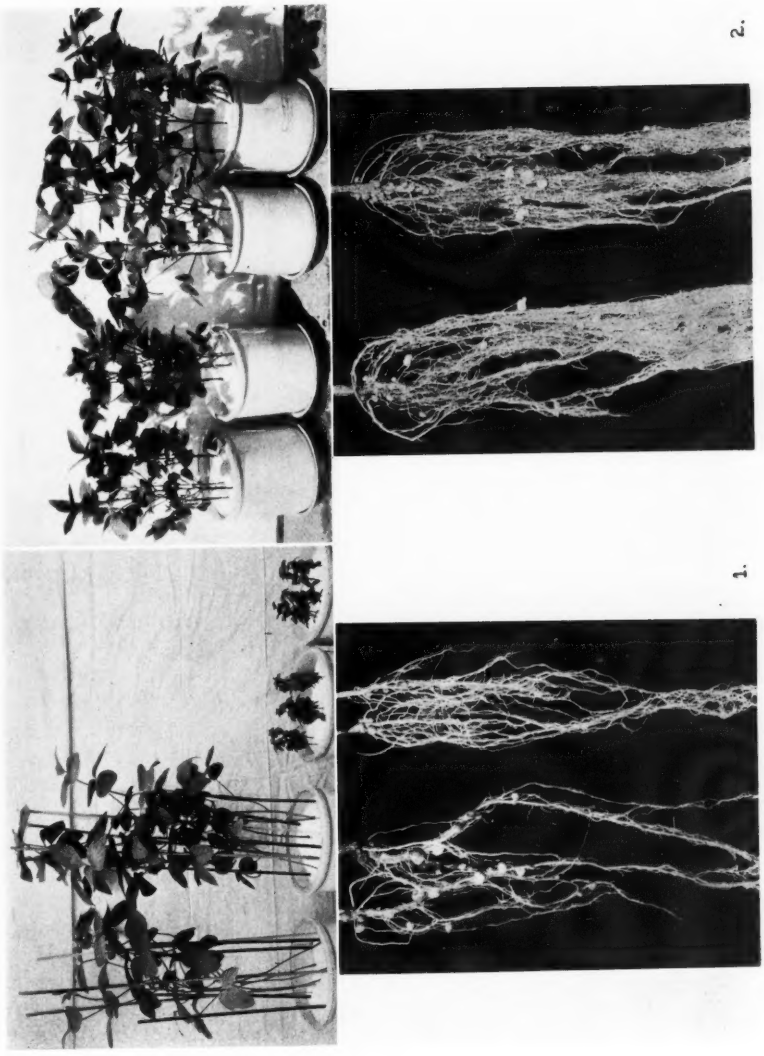
(36) WILSON, P. W., AND PETERSON, W. H. 1931 The energetics of heterotrophic bacteria. *Chem. Rev.* 8: 427–480.

PLATE 1

PLATE 1

FIG. 1. Tops and roots of plants from Experiment 2 at time of third harvest, plants 77 days old. Long day plants, left; short day plants, right.

FIG. 2. Tops and roots of plants from Experiment 4 at time of second harvest, plants 77 days old. Tops, unshaded plants, left; shaded plants, right. Roots, shaded plants, left; unshaded plants, right.



ORGANIZATION OF THE AMERICAN SECTION OF THE INTERNATIONAL SOCIETY OF SOIL SCIENCE

At a special meeting held directly following the banquet of the American Soil Survey Association on the evening of November 22, 1934, an American Section of the International Society of Soil Science was organized. The brief Constitution appended was unanimously adopted, and the following officers were elected: Dr. W. P. Kelley, of California, president; Dr. G. W. Conrey, of Ohio, vice-president; and Dr. A. G. McCall, of Washington, D. C., secretary-treasurer.

Dr. S. A. Waksman, of New Brunswick, New Jersey, and Dr. M. F. Morgan, of New Haven, Connecticut, were appointed a committee on arrangements for those planning to attend the International Congress of Soil Science to be held at Oxford, England, July 30 to August 7.

Members of the International Society of Soil Science are urged to affiliate with the American Section by sending their applications and dues to the Secretary-Treasurer, Dr. A. G. McCall, Bureau of Chemistry and Soils, Washington, D. C.

CONSTITUTION FOR THE AMERICAN SECTION, INTERNATIONAL SOCIETY OF SOIL SCIENCE

Adopted November 22, 1934, at Washington, D. C.

1. The *American Section of the International Society of Soil Science* shall be composed of those members in good standing in the International Society of Soil Science.

2. *Purpose:* The purpose of the Section will be to serve as a medium of expression for the American Members of the International Society, and as an organization through which these members can transact necessary business.

3. *Officers:* The officers shall be a President, Vice-President and a Secretary-Treasurer. They shall be elected at the annual meeting and shall serve for terms of one year, or until their successors are elected and assume office. The officers shall constitute the Executive Committee.

4. *Duties:* The duties of the officers shall be those that usually pertain to the respective offices. The duties of the Executive Committee shall be to determine policies and programs for the Section, and present their findings at the annual meetings for ratification or rejection. Between meetings of the Society, the Executive Committee shall act for the Society in matters of business.

5. *Meetings:* A business meeting shall be held annually, at such time and place as may be designated by the Section; or in case of no decision by the Section, on call of the Executive Committee.

6. *Dues:* Dues of the International Society of Soil Science are set by that body and are payable to the officers designated by that body. Dues in the American Section of the International Society shall be fifty cents per year. Annual dues may be waived or omitted for any year by decision of the Executive Committee if, in their judgment, the treasury has sufficient funds to carry on the functions of the Society without payment of dues.

THIRD INTERNATIONAL CONGRESS OF SOIL SCIENCE

The Third International Congress of Soil Science will be held in Oxford, July 30–August 7, under the presidency of Sir John Russell, Director of the Rothamsted Experimental Station, England. The two previous congresses of the series, held in Washington in 1927 and in Leningrad and Moscow in 1930, were notable for the exceptionally international character of the personnel and of the discussions. The forthcoming Congress will meet as a whole in six plenary sessions, at which a general survey of recent advances in every branch of soil science will be made, and it will also work in sections of commissions dealing specifically with (1) soil physics, (2) soil chemistry, (3) soil microbiology, (4) soil fertility, (5) soil classification, and (6) soil technology. Three subcommissions will discuss problems relating to alkali, forest, and peat soils. A 16-day excursion round Great Britain, leaving Oxford immediately after the Congress and terminating in Cambridge on August 23, has also been arranged for the benefit of members wishing to obtain first-hand knowledge of British agriculture and soils.

Every member of the Congress will receive a copy of the official transactions, including the full text of papers read at the plenary sessions, and detailed reports of the discussions at the Commission sessions. The cost of the transactions will be included in the Congress fee ($10), payment of which will also entitle members to attend all meetings, receptions, etc., held in connection with the Congress. College accommodation during the Congress can be reserved through the Organizing Committee. Those who are planning to take part in the excursion must deposit a registration fee of $10 before June 30. Intimation of attendance at the Congress should be sent as soon as possible to the Secretary of the Organizing Committee, Mr. G. V. Jacks, Imperial Bureau of Soil Science, Harpenden, England, from whom all further information may be obtained.

The Committee on Arrangements has determined that the approximate cost, for American delegates and those accompanying them, of the Congress and excursion, including Tourist transportation from New York and return to New York, will be about $380, with a reduction to members of the International Society (about $20). The committee recommends the following accommodations:

French Liner, *Ile de France*, leaving New York on July 20 and arriving at Plymouth on July 26, thus allowing three days to be spent in London before the Congress. Return on the *Champlain*, which leaves Southampton on

August 24. However, the members have the privilege of returning on other steamers (*Champlain*, August 7[1]; *Normandie*, August 14[2]; *Ile de France*, August 21[2]; *Normandie*, August 28[2]; *Lafayette*, September 4[1]; *Ile de France*, September 11[2]) from either Southampton or Havre. The U. S. Revenue Tax of $5 must be added to the above quotation. It may be noted that railroad transportation from Plymouth to London as well as from London to Southampton will be provided. Members desiring to continue to the Botanical Congress in Amsterdam (September 2 to September 7) may do so without additional transportation expense, railroad tickets being provided from London to Amsterdam, from Amsterdam to Paris, and from Paris to Havre.

The American Shipper, of the U. S. Lines leaves New York on July 20 and arrives at Liverpool on July 29. Accommodation Tourist Class on this steamer will be about $40 less than the above quotation for the round trip, but no transportation from and to Liverpool will be provided. One may return from London on the *American Merchant* leaving August 30.

Everyone contemplating going from the United States to the Congress is urged to write immediately to one of the members of the committee. It is understood that the above quotation will include only the Congress, excursion, and actual transportation and will not include board and lodging and incidental expenses en route to the Congress from Plymouth and after the excursion. It is also understood that the quotation applies only if at least 25 members travel in one group going from New York. The committee will make arrangements for reservations in line with the above plans.

[1] Slight reduction in above rate.
[2] Slight addition to above rate.